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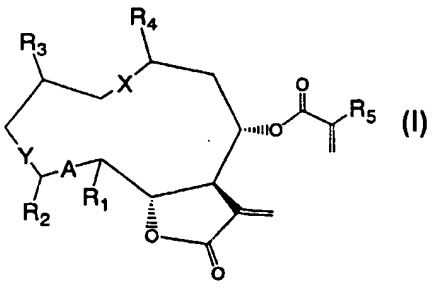
(54) Title: A SESQUITERPENOIC COMPOUND AND PHARMACEUTICAL FORMULATIONS COMPRISING THE SAME



(57) Abstract: The objective of the present invention is to provide a pharmaceutically useful compound which is effective against drug resistant protozoans, particularly to leishmania. The objective can be achieved by using a sesquiterpenoic compound shown in formula (I), or a derivative thereof.

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DESCRIPTION

A sesquiterpenoic compound and pharmaceutical formulations comprising the same

Technical Field

The present invention relates to a germacranolide-type or guaianolide-type sesquiterpenoic compound and pharmaceutical formulations, particularly antiprotozoal agents, comprising the same.

Background Art

As chemotherapeutic agents against infectious diseases caused by protozoans, a large number of agents, including quinine used against malaria, have been developed. However, every time a protozoan develops resistance to an agent, a new specific agent is required. Especially, in respect of malaria, the achievement of agent resistance by a protozoan causes a serious problem.

Leishmaniasis is a parasitic disease, which is caused by a protozoan, leishmania and appears specifically in tropical regions including South America, and it is one of the Six tropical diseases designated by WHO. The total number of patients in Africa, Middle East, Central and South America and Asia is approximately twelve million, and four hundred thousand people become newly infected with this disease every year. The infection route of this disease is a blood sucking insect, sandfly (*Phlebotomus*). When a sandfly sucks blood from the body, leishmania parasitizing in the sandfly infests into a human body, thereby establishing an infection. Leishmania is comprised of 4 complexes: Leishmania donovani, L. tropica, L. mexicana and L. braziliensis. The exposed morbidity is different for each of the 4 complexes, but basically the cause of the onset of this disease is that the protozoan parasitizes to a macrophage existing in each organ or locality of a patient. Viscerotonic leishmaniasis

is developed by the parasite of L. donovani to a macrophage or a reticuloendothelial cell existing in liver, spleen, bone marrow and the like. The predominant symptom is the overgrowth of liver and spleen, anaemia, the reduction of leucocytes, the onset of fever and the swelling of lymph glands. Cutaneous leishmaniasis is divided into old-world type and new-world type. The old-world type cutaneous leishmaniasis is developed by L. tropicana, whereas the new-world type cutaneous leishmaniasis is developed by L. mexicana. Both types are developed by the parasite of a protozoan to a macrophage in skin and subsequently form a cutaneous ulcer. L. braziliensis usually develops mucosal-cutaneous leishmaniasis creating a lesion in mucous membrane and skin, but some of them may develop cutaneous leishmaniasis. A protozoan, leishmania has a variety of species. Further, from the viewpoint of immunology, even though the morbidity is similar, their antigenicities are different depending on regions. This makes the development of vaccine difficult, and chemotherapy is highly required.

Presently, for the treatment of leishmaniasis, pentavalent antimony agents (the trade name: Pentostam, Glucantime) are used as the agents of first choice. When these antimony agents are not effective, Pentamidine, Amphotericin B and so on are used instead, but these agents have lower efficacy than antimony agents do. However, since the antimony agents may have strong side effect after the administration, a medical doctor's care is needed for their use. Moreover, the high price of antimony agents causes another problem. Furthermore, some kinds of protozoans have already developed resistance to the antimony agents, and so a novel, low-priced and safe therapeutical agent with fewer side effects is desirable.

Summary of the Invention

With regard to the application of chemotherapy to protozoal infections, the achievement of drug resistance by protozoans is the most serious problem. A typical example includes falciparummalaria's drug resistance. Although some therapeutical agents have been developed for this kind of malaria up till now, the protozoan has

become resistant to those agents and this infectious disease is still pervasive all across the world. The object of the present invention is to provide a pharmaceutically useful compound which is effective against drug resistant protozoans, particularly to leishmania.

The present inventors have found that the sesquiterpenoic compounds shown in formulas I to IV set forth later or derivatives thereof are useful as pharmaceuticals, particularly as antiprotozoal agents.

In addition, the present inventors have found the use of the sesquiterpenoic compounds shown in formulas I to IV or derivatives thereof as antiprotozoal agents, that is to say, they have found the use of antiprotozoal agents comprising the above sesquiterpenoic compounds or derivatives thereof; the use of the above sesquiterpenoic compounds or derivatives thereof for the production of antiprotozoal agents; or the use of the above sesquiterpenoic compounds or derivatives thereof for the treatment of protozoal infections.

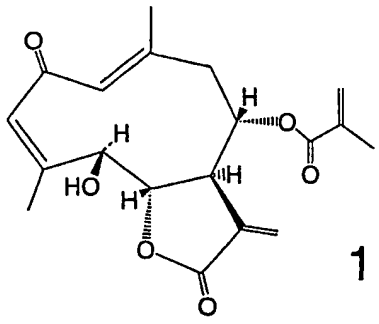
Furthermore, the present inventors have found the sesquiterpenoic compounds shown in formulas 5, 6, 7, 9, 10, 14 and 17 or derivatives thereof, and their utility as a pharmaceutical formulations.

Moreover, the present inventors have found the use of the sesquiterpenoic compounds shown in formulas 1 to 7, 9, 10, and 13 to 18 or derivatives thereof as antiprotozoal agents, that is to say, they have found the use of antiprotozoal agents comprising the above sesquiterpenoic compounds or derivatives thereof; the use of the above sesquiterpenoic compounds or derivatives thereof for the production of antiprotozoal agents; or the use of the above sesquiterpenoic compounds or derivatives thereof for the treatment of protozoal infections.

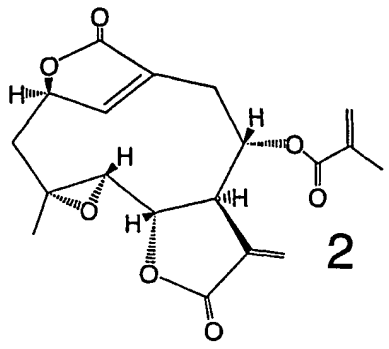
Furthermore, the present inventors have found that a composition comprising, as active ingredients, the compounds of formulas I to IV obtained by extracting from composite plants, is useful as a pharmaceutical formulation, particularly as an antiprotozoal agent.

Disclosure of the Invention

Having purified and isolated compounds shown in the following formulas 1 to 7 from a Peruvian composite plant, *Elephantopus* mollis H.B.K., the present inventors have found that all of these compounds show strong anti-leishmanial activity, and reported on it at the 46th Annual Meeting of the Japanese Society of Pharmacognosy held on September 17, 1999 (Hiroyuki Fuchino, Tatsuo Koide, Marii Takahashi, Setsuko Sekita, Motoyoshi Satake, *the 46th Annual Meeting of the Japanese Society of Pharmacognosy Abstract Papers*, p.150 (1999)) and 22nd IUPAC International Symposium on the Chemistry of Natural Products held on September 4, 2000 (*22nd IUPAC International Symposium on the Chemistry of Natural Products Abstract Papers*, PPA-035 (2000)).



1



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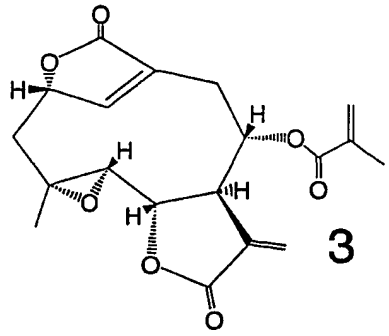

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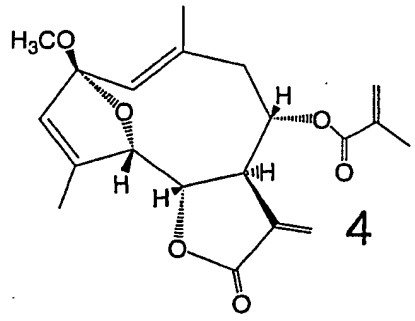

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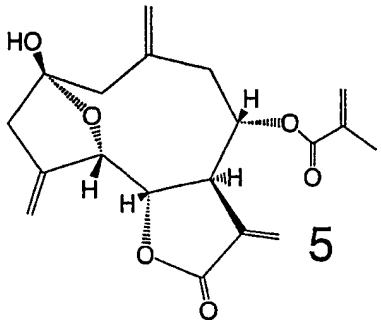

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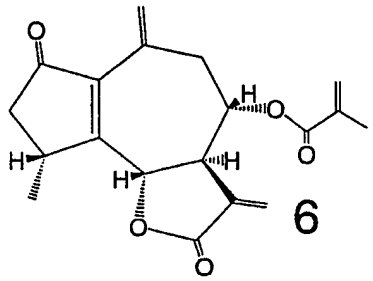

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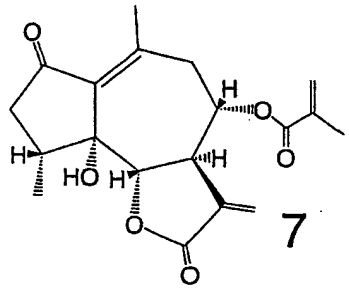

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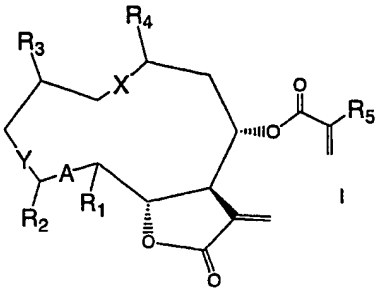
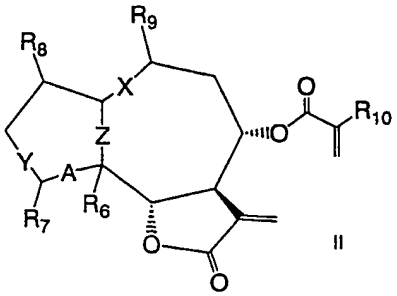
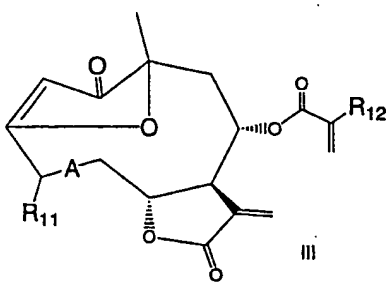
From subsequent studies, the present inventors conceived that usefulness as an antiprotozoal agent, particularly an anti-leishmanial agent could be obtained, not only where the agent comprises any one of the structures of compounds 1 to 7, but also where it comprises a germacran-type or guaian-type sesquiterpenoic compound having specific substituents shown in the following formulas I, II and III:



I



II

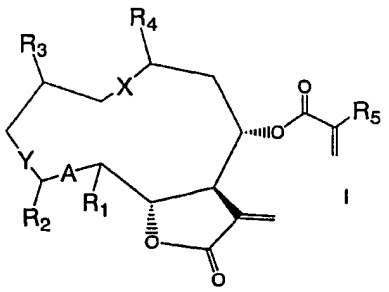


III

, thereby they have already applied for a patent on February 10, 2000 under Japanese Patent Application No. 2000-33232.

Now, the present inventors, claiming the right of priority regarding this Japanese patent application, file the present application with some further examples providing support for this conception. Furthermore, the present application comprises a new finding, that compounds shown in the following formulas IIa (the part comprised in formula II is excluded) and IV are also useful as antiprotozoal agents, particularly anti-leishmanial agents, which was not disclosed in Japanese Patent Application No. 2000-33232.

That is to say, the present invention relates to a sesquiterpenoic compound shown in the following formula I, IIa, IIIb or IV, or a derivative thereof:



I

wherein

-X-, -Y- and –A- represent a single bond or a double bond,

R_1 is H, OH, =O, an -O- bond to C at position 2 or 4, or a single bond to C at position 10,

R_2 is methyl or methylene,

R_3 is H, OH or =O,

R_4 is methyl or methylene,

R_5 is methyl, and

R_3 and R_4 may together form –O-CO-;

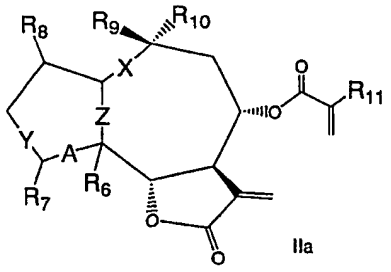
with the exception that the following compounds (1) to (4) are excluded from the compounds shown in the above formula I or derivatives thereof:

(1) a compound wherein –X- and –Y- represent a double bond, -A- represent a single bond, each of R_2, R_4 and R_5 is methyl, R_3 is =O, R_1 is OH, acetyloxy, =O, or p-Br-benzenesulfonyloxy;

(2) a compound wherein –X- represents a double bond, -Y- and –A- represent a single bond, R_1 is an –O- bond to C at position 4, R_2 and R_5 are methyl, R_3 and R_4 are respectively OH and methyl, or R_3 and R_4 together form –O-C(O)- or a methylacetal or ethylacetal derivative thereof;

(3) a compound wherein –X- and –A- represent a double bond, -Y- represents a single bond, R_1 is H, R_2 and R_5 are methyl, R_3 and R_4 together form –O-C(O)-; and

(4) a compound wherein –X- and –Y- represent a double bond, -A- represents a single bond, R_1 represents a –O-bond to C at position 2, R_2, R_4 and R_5 are methyl, R_3 is OH, methoxyl or ethoxyl;



IIa

wherein

-X-, -Y-, -Z- and –A- represent a single bond or a double bond,

R_6 does not exist or represents OH,

R_7 is methyl or methylene,

R_8 is OH or =O,

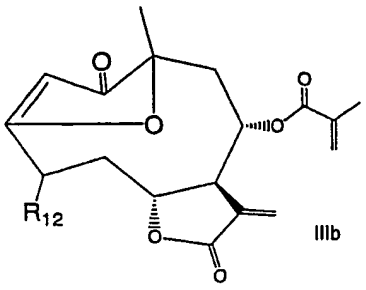
R_9 and R_{10} do not exist or represent methyl, methylene or OH, and

R_{11} represents methyl;

with the exception that the following compounds (1) and (2) are excluded from the compounds shown in the above formula IIa or derivatives thereof:

(1) a compound wherein each of –X-, -Z- and –A- represents a single bond, -Y- represents a double bond, R_6 is OH, each of R_7, R_{10} and R_{11} is methyl, R_8 is =O, and R_9 is OH;

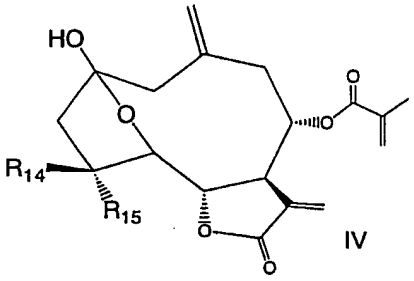
(2) a compound wherein -A- and –Z- represent a single bond, -X- and –Y- are a double bond, R_6 is OH, R_7 and R_{11} are methyl, R_8 is =O, and R_9 and R_{10} together form methyl;



IIIb

wherein

R_{12} is methyl or methylene; or

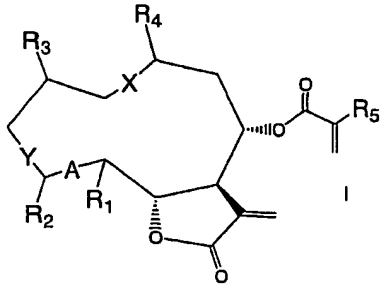


IV

wherein

R_{14} and R_{15} respectively represent methyl or OH, and alkoxyl.

Furthermore, the present invention relates to a pharmaceutical formulation comprising a sesquiterpenoic compound shown in the following formula I, IIa, IIIc or IV, or a derivative thereof:



I

wherein

-X-, -Y- and –A- represent a single bond or a double bond,

R_1 is H, OH, =O, an -O- bond to C at position 2 or 4, or a single bond to C at position 10,

R_2 is methyl or methylene,

R_3 is H, OH or =O,

R_4 is methyl or methylene,

R_5 is methyl, and

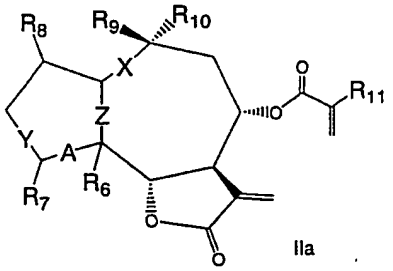
R_3 and R_4 may together form –O-CO-;

with the exception that the following compounds (1) to (3) are excluded from the compounds shown in the above formula I or derivatives thereof:

(1) a compound wherein –X- and –Y- represent a double bond, -A- represents a single bond, each of R_2, R_4 and R_5 is methyl, R_3 is =O, R_1 is OH, acetyloxy, =O or p-Br-benzenesulfonyloxy;

(2) a compound wherein –X- represents a double bond, -Y- and –A- represent a single bond, R_1 is an –O- bond to C at position 4, R_2 and R_5 are methyl, R_3 and R_4 are respectively OH and methyl, or R_3 and R_4 together form –O-C(O)- or a methylacetal or ethylacetal derivative thereof;

(3) a compound wherein –X- and –A- represent a double bond, -Y- represents a single bond, R_1 is H, R_2 and R_5 are methyl, R_3 and R_4 together form –O-C(O)-;



IIa

wherein

-X-, -Y-, -Z- and –A- represent a single bond or a double bond,

R_6 does not exist or represents OH,

R_7 is methyl or methylene,

R_8 is OH or =O,

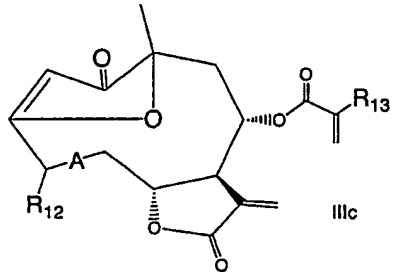
R_9 and R_{10} do not exist or represent methyl, methylene or OH, and

R_{11} represents methyl;

with the exception that the following compounds (1) and (2) are excluded from the compounds shown in the above formula IIa or derivatives thereof:

(1) a compound wherein each of –X-, -Z- and –A- represents a single bond, -Y- represents a double bond, R_6 is OH, each of R_7, R_{10} and R_{11} is methyl, R_8 is =O, and R_9 is OH;

(2) a compound wherein -A- and –Z- represent a single bond, -X- and –Y- represent a double bond, R_6 is OH, R_7 and R_{11} are methyl, R_8 is =O, and R_9 and R_{10} together form methyl;



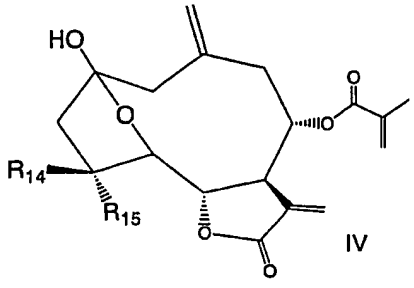
IIIc

wherein

-A- represents a single bond or a double bond,

R_{12} is methyl or methylene, and

R_{13} is methyl; or



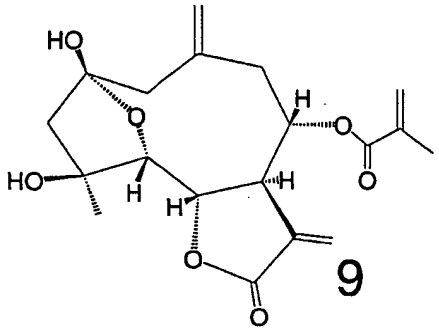
IV

wherein

R_{14} and R_{15} respectively represent methyl or OH, and alkoxyl.

Furthermore, the present invention relates to the use of a sesquiterpenoic compound shown in formula I, IIa, IIIc or IV or a derivative thereof, as an antiprotozoal agent, that is to say, the use of antiprotozoal agents comprising the above sesquiterpenoic compound or a derivative thereof; the use of the above sesquiterpenoic compound or a derivative thereof for the production of antiprotozoal agents; or the use of the above sesquiterpenoic compound or a derivative thereof for the treatment of protozoal infections.

Moreover, the present invention relates to a pharmaceutical formulation comprising the compound shown in the above formula 5, 6 or 7, or the following formula 9, 10, 14 or 17, or a derivative thereof:



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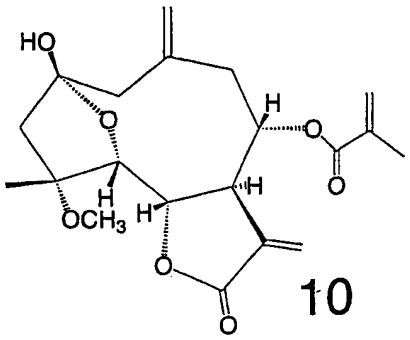

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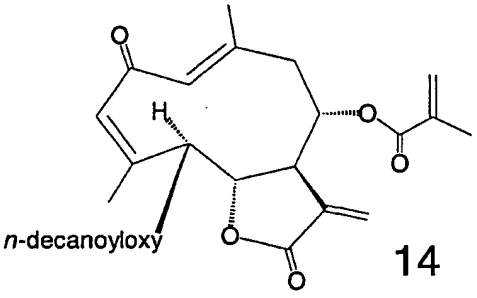

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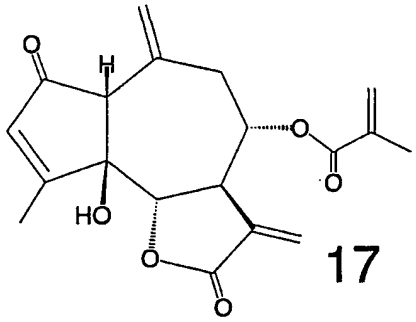

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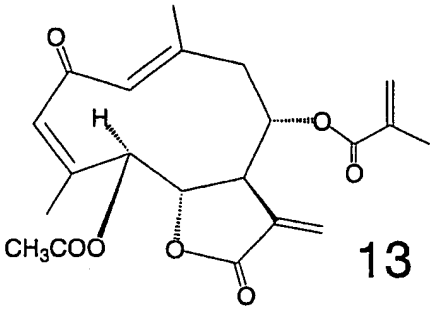
Further, the present invention relates to the use of a sesquiterpenoic compound shown in the above formula 1 to 7, 9, 10, 14 or 17, or in the following formula 13, 15, 16 or 18, or a derivative thereof, as an antiprotozoal agent, that is to say, the use of antiprotozoal agents comprising the above sesquiterpenoic compound or a derivative thereof; the use of the above sesquiterpenoic compound or a derivative thereof for the production of antiprotozoal agents; or the use of the above sesquiterpenoic compound or a derivative thereof for the treatment of protozoal

infections.



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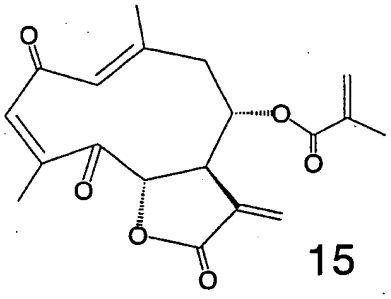

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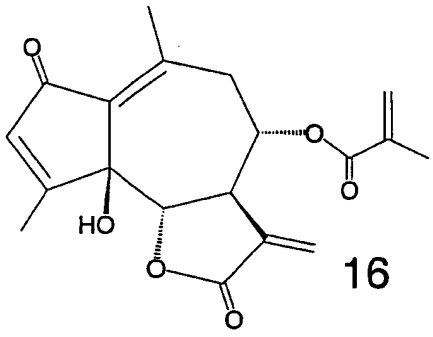

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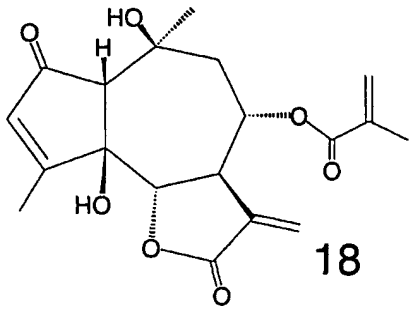


18

Furthermore, the present invention relates to a pharmaceutical formulation which consists of a composition comprising, as active ingredient, the compound of the above formula I, IIa, IIIc or IV obtained by extraction from composite plants.

Moreover, the present invention relates to the use of the compound of the above formula I, IIa, IIIc or IV obtained by extraction from composite plants, as an antiprotozoal agent, that is to say, the use of antiprotozoal agent comprising the above composition or a derivative thereof; the use of the above composition or a derivative thereof for the production of antiprotozoal agents; or the use of the above composition or a derivative thereof for the treatment of protozoal infections.

The derivatives of the sesquiterpenoic compound of the present invention include those of hydroxyl and carbonyl.

The derivatives of the compound shown in formula 1 include those of hydroxyl and carbonyl, and the derivatives of the compounds shown in formulas 2 and 3 include acetal derivatives of carbonyl in a lactone ring.

The derivatives of the compound shown in formula 5 include those of hydroxyl, the derivatives of the compound shown in formula 6 include those of carbonyl at position 2, the derivatives of the compound shown in formula 7 include those of hydroxyl and carbonyl at position 2, and the derivatives of the compounds

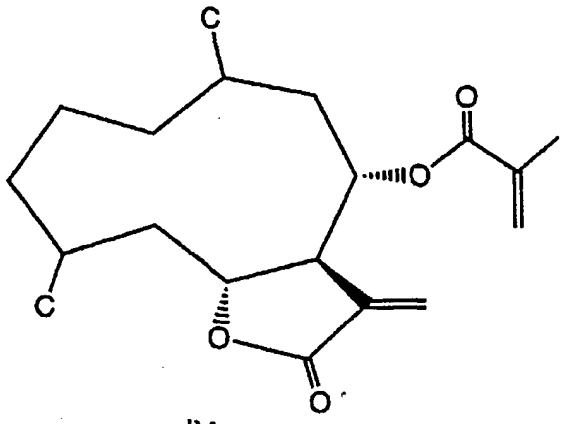
shown in formulas 9 and 10 include those of hydroxyl.

The preferable derivatives of hydroxyl include alkoxyl, acyloxy, silyloxyl, etc.

In the present invention, examples of alkoxyl include methoxyl, ethoxyl, propyloxyl, butyloxyl, etc. Similarly, examples of acyloxy include acetyloxy, propionyloxy, butyryloxy, alkylcarbonyloxy such as valeryloxy, and arylcarbonyloxy such as benzoyloxy. Examples of silyloxyl include trimethylsilyloxyl.

The derivatives of carbonyl include acetal, hydrazone or oxime.

As it is clear from the chemical structure formulas, the sesquiterpenoic compound of the present invention characteristically has a common basic skeleton shown in the following formula A:



式A .

It has been reported that sesquiterpenoic compounds shown in the above formulas 1 to 4 were obtained from *Elephantopus*. Especially, Elephontopus mollis H. B. K. (which is called "lingua de vaca" locally) is a composite plant distributed in Central and South America, and locally is used as a decoction to make the body analeptic or to treat dyshidrosis, coughing, bronchitis and nephrolithiasis. It is also

used as a strapping for dermatosis or elephantiasis. It has also been reported that the compounds shown in formulas 1 and 2 have antitumoral activity (S. Kupchan *et al*, *J .Am. Chem. Soc.*, vol. 88, p.3674 (1966), P. P. But *et al*, *Plant. Med.*, vol. 62, p.474 (1996), J. Jakupovic *et al*, *Phytochemistry*, vol. 26, p.1467 (1987), K. H .Lee *et al*, *J. Chem. Soc. Chem. Comm.*, p.476 (1973), McPhail, A. T. *et al*, *Tetrahedron Lett.*, p.2739 (1974), Watson, W. H. *et al*, *Acta Crystallogr., Sect.B*, vol.38, p.511 (1982), Haruna, M. *et al*, *J. Nat. Prod.*, vol. 48, p.93 (1985), Zhang, D. *et al*, *Phytochemistry*, vol.25, p.899 (1987), T. Kurokawa *et al*, *Tetrahedron Lett.*, p.2863 (1970), S. Banerjee *et al*, *Planta Medica*, p29 (1986), T. R. Govindachari *et al*, *Indian J. Chem.*, vol.10, p272 (1972), K. H. Lee *et al*, *J. Pharm. Sci.*, vol.64, p.1077 (1975), S. Kupchan *et al*, *J. Med. Chem.*, vol.14, p.1147 (1971), K. H. Lee *et al*, *J. Pharm. Sci.*, vol.68, p.1050 (1980), K. H. Lee *et al*, *J. Pharm. Sci.*, vol.64, p.1572 (1975), T. Hayashi *et al*, *Phytochemistry*, vol.26, p.1065 (1987), T. Hayashi *et al*, *J. Nat. Prod.*, vol.62, p.302 (1999), M. I. Ybarra *et al*, *Phytochemistry*, vol.29, p.2020(1990), P. P-Y But *et al*, *Phytochemistry*, vol.44, p.113 (1997), *Phytochemistry*, vol.21, p.1173 (1982), A.T. McPhail, *et al*, *J. Chem. Soc., Perkin Trans.*, vol.2, p.1313 (1972), F.Bohmann *et al*, *Phytochemistry*, vol.20, p.263 (1981), L. B. de Silva *et al*, *Phytochemistry*, vol.21, p.1173 (1982)). However, the antiprotozoal activity of the above-stated compounds is not described in these publications.

As stated above, after the present inventors had succeeded in purifying and isolating the compounds shown in formulas 1 to 7 from a Peruvian composite plant, *Elephantopus* mollis H.B.K., they have reported on the strong anti-leishmanial activity of these compounds (Hiroyuki Fuchino, Tatsuo Koide, Marii Takahashi, Setsuko Sekita, Motoyoshi Satake, *the 46th Annual Meeting of the Japanese Society of Pharmacognosy Abstract Papers*, p.150 (1999) and *22nd IUPAC International Symposium on the Chemistry of Natural Products Abstract Papers*, PPA-035 (2000)).

Furthermore, among the compounds on which the present inventors clarify the

usefulness as an antiprotozoal agent in the present application, a compound shown in the following formula 8 is already known (Vichnewski, W. *et al*, *Phytochemistry*, vol.15, p.1775 (1976), vol.21, p.464 (1982), Chawdhury, P. K. *et al*, *J. Org. Chem.*, vol.45, p.4993 (1980), Herz. W. *et al*, *J. Org. Chem.*, vol.47, p.2798 (1982)). However, regarding this compound also, its antiprotozoal activity is still unknown. There are provided some reports regarding the relative compounds (*Planta Medica*, vol.58, p.474 (1992)), but their antiprotozoal activity is also unknown.

The compounds shown in formulas 9 and 10 are novel ones obtained by purifying and isolating from *Elephantopus* mollis H.B.K. of Brazilian origin by the present inventors.

Among the compounds shown in formulas 13 to 18, the compounds of formulas 13 and 15 are known (Lee KH, Furukawa H, Kozuka M, *Chemical Communication, J. Chem. Soc.*, p.476-477 (1973); Lee KH, Ibuka T, Furukawa H, Kozuka M, Wu RY, Hall IH, Huang HC., *J. Pharm. Sci.*, vol.68, p.1050-1056 (1980)), and each of two compounds have been obtained by derivation from the compound of formula 1.

The compound shown in formula 14 is novel.

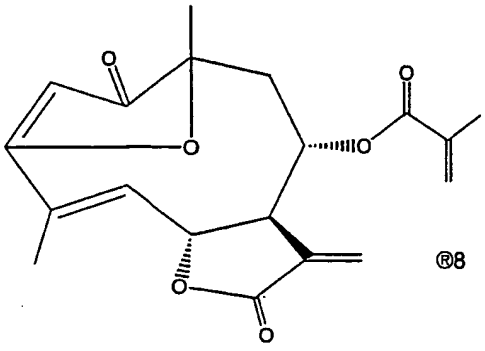
The compounds shown in formulas 16 to 18 have been obtained from the compound of formula 1 according to a known production method (Lee KH, Ibuka T, Furukawa H, Kozuka M, Wu RY, Hall IH, Huang IC., *J. Pharm. Sci.*, vol.68, p.1050-1056 (1980). The compounds of formulas 16 and 18 are known, but the compound of formula 17 is novel.

At a meeting of the Pharmaceutical Society of Japan held on March 30, 2000, the present inventors reported that the compounds shown in formulas 13 to 18 have

anti-leishmanial activity (Hiroyuki Fuchino *et al.*, *the Pharmaceutical Society of Japan the 120th Annual Meeting Abstract Papers*, vol.2, p.125 (2000)). It is the present inventors who have clarified for the first time the usefulness of the compounds of formulas 13 to 18 as antiprotozoal agents.

As stated above, among the group of compounds, the usefulness of which, as an antiprotozoal agent, particularly an anti-leishmanial agent has been clarified by the present inventors, there exist some novel compounds of which usefulness is completely unknown. On the other hand, there are some other known compounds, and the use of these compounds other than as an antiprotozoal agent has been reported. However, the present inventors have found for the first time that these are useful as anti-leishmanial agent, which could not be expected from previous reports.

The specific compounds having the antiprotozoal activity of the present invention include the compounds shown in the following formulas 8, 8-1, 11, 11-1 and the above formulas 13 to 18, as well as the compounds shown in the above formulas 1 to 7, 9 and 10:



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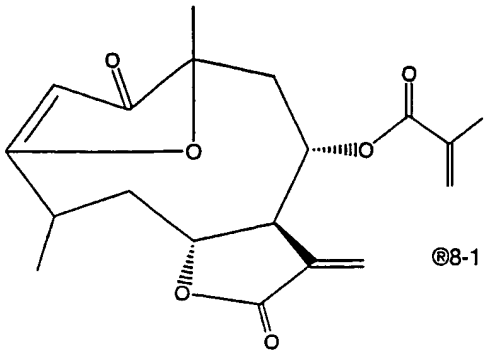
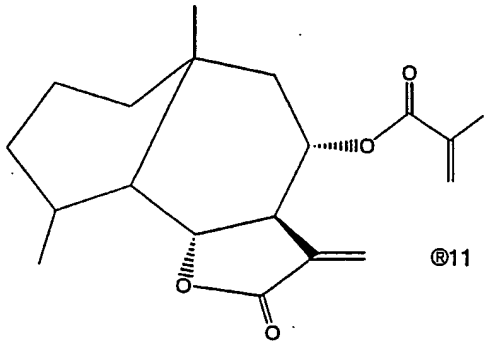
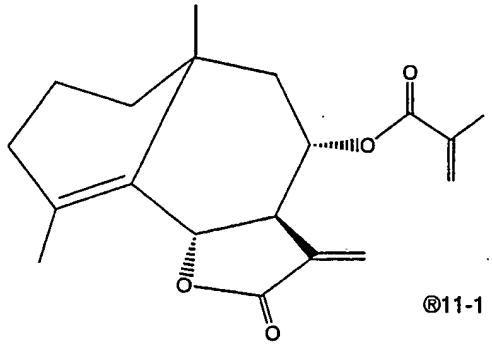


®8-1



®11



®11-1

The method of producing the compound of the present invention is described below.

The sesquiterpenoic compounds shown in formulas I-IV or the derivatives of the present invention can be obtained by purifying and separating from plants e.g. composite plants, or production from compounds which are extracted from plants. As

stated above, the sesquiterpenoic compounds shown in formulas 1 to 4 or formula 8 are publicly known. The production method of the compounds of formulas 1 to 7, as stated above, has already been reported by the present inventors (Hiroyuki Fuchino *et al., the 46th Annual Meeting of the Japanese Society of Pharmacognosy Abstract Papers*, p.150 (1999) and Hiroyuki Fuchino *et al., 22nd IUPAC International Symposium on the Chemistry of Natural Products Abstract Papers*, PPA-035 (2000)). And the production method of the compounds of formulas 13 to 18 has also been reported (Hiroyuki Fuchino *et al., the Pharmaceutical Society of Japan the 120th Annual Meeting Abstract Papers*, vol.2, p.125 (2000).

The plants from which the sesquiterpenoic compounds are extracted include *Elephantopus* or *Helianthus*, *Calea*, and *Vanillosmopsis*.

The extraction and purification from plants can be carried out according to the following publicly known method. The entire or the terrestrial part of plants used as a material is collected at appropriate period, is subjected to air-drying and the like, and then is extracted to obtain the extract. The juice squeezed out of plants can also be used as an extract material.

Sesquiterpenoic compounds can be extracted from the above dried plant body according to known procedures. The extract can be obtained from natural extract materials, or crushed or macerated ones, using a solvent. This process can be carried out according to a batch system or continuous system extraction. As an extraction solvent, organic solvents such as dichloromethane, chloroform, hexane, etc. can be used. Generally, this extraction procedure is carried out at room temperature.

From crude extract obtained according to the above procedure, sesquiterpenoid is purified and separated. Before the purification and separation, insoluble residue can be removed from the crude extract by filtration, centrifugation and

so on. A more concentrated extract is obtained by removing solvent from the extract, and then the concentrated extract is purified and separated. For the purification and separation, known methods including silica gel or activated carbon column chromatography, gel filtration chromatography and liquid chromatography can be used singly or in combination.

Alkoxyl derivatives can be produced by alkylating with known alkylating agents such as alkyl halide. For example, methoxyl derivatives can be produced using iodomethane according to the method described in N. Finch, J. Fitt, I.H.S.Hsu, *J. Org. Chem.*, vol.40, p.206 (1975) or an equivalent method.

Acyloxy derivatives can be produced by acylating with known acylating agents such as acid anhydride or acid chloride.

Acetoxyl derivatives can be produced using acetic anhydride according to the method described in H. Weber, H.G. Khorana, *J. Mol. Biol.*, vol.72, p.219 (1972) or an equivalent method.

Silyloxyl derivatives can be produced by silylating with known silylating agents such as trialkylsilylchloride.

Triethylsilyloxyl derivatives can be produced using triethylsilylchloride according to the method described in E. J. Corey, H. Cho, C. Rucker, D.H. Hua, *Tetrahedron Lett.*, vol.22, p.3455 (1981) or an equivalent method.

Acetal derivatives can be produced using ethylene glycol according to the method described in C. H. Heasthcock, R. Ratcliffe, *J. Am. Chem. Soc.*, vol.93, p.1746 (1971) or an equivalent method.

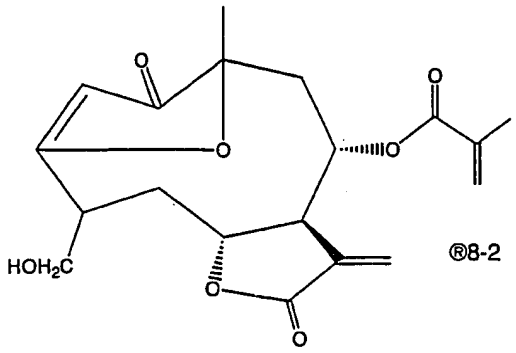
Hydrazone derivatives can be produced according to the method described in G. R. Newkome, D. L. Fishel, *Org. Synth.*, vol.50, p.102 (1970) or an equivalent method.

Oxime derivatives can be produced according to the method described in R. V. Stevens, F. C. A. Gaeta, D. S. Lawrence, J. Am. Chem. Soc., vol. 105, p.7713 (1983) or an equivalent method.

The sesquiterpenoic compounds shown in formulas 1 to 7, 9 and 10 can be obtained by extraction from a Peruvian composite plant, *Elephantopus* mollis H. B. K. (which is locally called "lingua de vaca"), and then purifying the extract to separate. As a method of purifying and separating the sesquiterpenoic compound, silica gel column chromatography, gel filtration chromatography, high performance liquid chromatography and the like can be used.

The sesquiterpenoic compound shown in formula IIIc (or IIIb) or the derivatives can be produced by the following process:

The compounds shown in formulas 8 and 8-1 can be produced from the compound shown in formula 8-2 set forth below:



®8-2

For example, the compound of formula 8 can be produced by iodinating hydroxymethyl at position C-5 of the compound of formula 8-2, and then deriving it to methyl using metallic reagent such as tributyltin hydride. The compound of formula 8-1 can be produced by reducing a double bond at position 4 of the compound of formula 8-2 by catalytic hydrogenation.

Iodination can be carried out according to the method described in P. J. Garegg, B. Samuelsson, *J. Chem. Soc. Chem. Commun.*, p.978 (1979) or an equivalent method. In addition, the conversion of methyl iodide into methyl can be carried out, for example, according to the methods described in S. J. Cristol, R. M. Sequeira, C. H. DePuy, *J. Am. Chem. Soc.*, vol. 87, p.4007 (1965) and G. Cardillo, M. Orena, S. Sandri, C. Tomasini, *J. Org. Chem.*, vol. 49, p.3951 (1984) or an equivalent method.

Furthermore, the compound of formula 8 can be derived to the compound of formula 8-1, for example, according to the method described in W.F. Bruce, J. O. Ralls, *Org. Synth., Coll.* vol. II, p.191 (1943) or an equivalent method.

The compound of formula 8-2 used as a material can be extracted from composite plants according to the methods described in W. Vichnewski, S. J. Sarti, B. Gilbert, W. Herz, *Phytochemistry*, vol.15, p.191 (1966) and W. Herz, G. Hogenauer, *J. Org. Chem.*, vol. 27, p.905 (1962) or an equivalent method.

The compound of formula 13 can be produced according to the method described in Lee KH, Furukawa H, Kozuka M, *Chemical Communication, J. Chem. Soc.*, p.476-477 (1973) and Lee KH, Ibuka T, Furukawa H, Kozuka M, Wu Ry, Hall IH, Huang HC., *J. Pharm. Sci.*, vol.68, p.1050-1056 (1980), or an equivalent method.

The compound formula 14 can be produced according the method described later or an equivalent method.

The compound of formula 15 can be produced according to the method described in Lee KH, Ibuka T, Furukawa H, Kozuka M, Wu Ry, Hall IH, Huang HC., *J. Pharm. Sci.*, vol.68, p.1050-1056 (1980), or an equivalent method.

The compounds of formula 16, 17 and 18 can be produced according to the method described in Lee KH, Ibuka T, Furukawa H, Kozuka M, Wu Ry, Hall IH, Huang HC., *J. Pharm. Sci.*, vol.68, p.1050-1056 (1980), or an equivalent method.

The sesquiterpenoic compounds of the present invention or these derivatives have antiprotozoal activity, particularly anti-leishmanial activity. So, these compounds can be used as antiprotozoal agents. As described later, when an acute toxicity test was carried out using the methanol extract obtained from *Elephantopus* mollis H.B.K. containing large amounts of sesquiterpenoid of the present invention, there was found no toxicity. Therefore, it is considered that the sesquiterpenoic compounds of the present invention or these derivatives do not have any serious toxicity problems.

The pharmaceutical formulation of the present invention can be administered both orally and parenterally, and the formulation can be processed into dosage forms suitable for each case. Where the present pharmaceutical formulation is administered orally, this can be applied as a tablet, pill, capsule, powder, granule, emulsion, solution, syrup and elixir. It is also preferable to be applied as an extract containing the above compound, which is an extract composition from plants. Furthermore, when the present pharmaceutical formulation is applied as a tablet, pill, capsule, powder or granule, it may contain additive agents such as an excipient, lubricant, bonding agent, disintegrating agent, stabilizer, solubilizer and the like. When the present pharmaceutical formulation is applied as an emulsion, solution, syrup or elixir, it can contain other additive agents such as an antiseptic as an adjuvant.

Where the present pharmaceutical formulation is administered parenterally, this can be applied as an injection, ointment, plaster, poultice, liniment, lotion, suppository and the like. When the present pharmaceutical formulation is applied as an injection, it may contain some additive agents such as a stabilizer, solubilizer, suspending agent, emulsifier, buffer agent, preservative, or other appropriate additive agents. Diluents may be added to the preparation to be dissolved before use. When it is applied as an aqueous injection, it may contain, e.g. distilled water for injection, physiological salt solution and Ringer's solution, as solvents. When it is applied as a nonaqueous injection, it may contain e.g. vegetable oil.

When the present pharmaceutical formulation is applied as an ointment, plaster and suppository, an appropriate base may be used. When it is applied as a poultice, liniment, lotion, it may contain a preservative or an aromatic. The liniment may contain water, ethanol, fatty oil, glycerin, soap, emulsifier, suspending agent or other appropriate additive agents. The lotion may contain the mixture of aqueous liquid and an appropriate solvent, emulsifier, suspending agent or the like. The poultice may contain appropriate essential oil ingredients.

The applied dosage of the present pharmaceutical formulation depends on morbidity, age, body weight and administration route, but when it is administered orally, in general, 1mg to 1,000mg per adult is administered one or several times per day. However, this applied dosage depends on patients' body condition, and the amount is not limited to the above range.

The Best Mode for Carrying Out the Invention

Example 1.

200g of dried *Elephantopus* mollis was extracted with 3L of dichloromethane. Solvent was removed from the obtained extract under reduced pressure to obtain concentrated extract. The obtained concentrated extract was independently purified

with silica gel column chromatography (developing solvent: the mixed solvent of chloroform and ethyl acetate); a gel filtration column chromatography (developing solvent: methanol) wherein Sephadex LH-20 (TM, Pharmacia Fine Chemicals) was used; and a high performance liquid chromatography (developing solvent: the mixed solvent of chloroform and ethyl acetate) wherein Aquasil (TM, Senshu Scientific Co., Ltd.) was used. As a result, compounds 1 to 7, 9 and 10 shown in the above formulas 1 to 7, 9 and 10 were obtained. Compounds 1 to 7 are germacran-type sesquiterpenoic compounds, and compounds 9 and 10 are guaian-type sesquiterpenoic ones. Both the physical properties and ^{1}H-NMR chemical shift values of compounds 1 to 7, 9 and 10 are shown below. Regarding the symbols used, s denotes singlet, d denotes doublet, dd denotes double doublet, ddd denotes double double doublet, t denotes triplet, m denotes multiplet, j (italic) denotes coupling constant, and Hz denotes Hertz, respectively.

Compound 1: achromatic spicula, fusing point 215-217 ℃ 、 ^{1}H-NMR (in dimethylsulfoxide-d_6) δ : 1.66 (3H, s), 1.90 (3H, s), 1.90 (3H, d, *J*=1.5Hz), 2.54 (1H, dd, *J*=11.6, 4.0Hz), 2.64 (1H, dd, *J*=11.6, 10.7Hz), 3.50 (1H, m), 4.16 (1H, d, *J*=3.1Hz), 5.24 (1H, m), 5.46 (1H, d, *J*=5.8Hz), 5.67 (1H, d, *J*=1.2Hz), 5.76 (1H, s), 5.87 (1H, t-like), 6.11 (1H, s), 6.56 (1H, s).

Compound 2: achromatic spicula, fusing point 260-264℃、 ^{1}H-NMR (in chloroform-d_1) δ : 1.31 (3H, s), 1.65 (1H, dd, *J*=14.3, 2.8Hz), 2.59 (1H, d, *J*=9.8Hz), 2.80 (1H, dd, *J*=14.3, 3.7Hz), 2.82 (1H, dd, *J*=12.2, 11.9Hz), 3.08 (1H, dd, *J*=12.2, 2.4Hz), 3.25 (1H, m), 4.32 (1H, m), 4.38 (1H, dd, *J*=9.8, 7.9Hz), 5.36 (1H, dd, *J*=3.7, 2.8Hz), 5.63 (1H, s), 5.65 (1H, d, *J*=3.4Hz), 6.10 (1H, s), 6.23 (1H, d, *J*=3.7Hz), 7.49 (1H, s).

Compound 3: achromatic oily substance, ^{1}H-NMR (in chloroform-d_1) δ : 1.48 (3H, s), 2.16 (1H, dd, *J*=15.3, 5.5Hz), 2.55 (1H, d, *J*=15.3Hz), 2.81 (1H, d, *J*=9.5Hz), 2.89 (1H, dd, *J*=12.5, 3.7Hz), 3.10 (1H, dd, *J*=12.5, 12.2Hz), 3.28 (1H, m), 4.31 (1H, dd, *J*=9.5, 9.5Hz), 4.55 (1H, m), 5.38 (1H, d, *J*=5.5Hz), 5.69 (1H, s), 5.73 (1H, d, *J*=3.1Hz), 6.16 (1H, s), 6.27 (1H, d, *J*=3.7Hz), 7.54 (1H, s).

Compound 4: achromatic oily substance, ^{1}H-NMR (in chloroform-d_1) δ : 1.74 (3H, s),

1.78 (3H, d, J=1.2Hz), 1.99 (3H,s), 2.23 (1H, dd, *J*=14.4, 4.4Hz), 3.15 (1H, m), 3.22 (3H, s), 3.73 (1H, dd, *J*=14.4, 2.1Hz), 4.66 (1H, dd, J=5.8, 3.7Hz), 5.23 (1H, m), 5.25 (1H, d, *J*=3.7Hz), 5.49 (1H, q, *J*=1.2Hz), 5.64 (1H, s), 5.71 (1H, s), 5.80 (1H, d, *J*=2.8Hz), 6.16 (1H, s), 6.32 (1H, d, *J*=3.1Hz).

Compound 5: achromatic oily substance, ^{1}H-NMR (in methanol-d_4) δ : 1.90 (3H, d, *J*=1.2Hz), 2.36 (1H, d, *J*=13.4Hz), 2.49 (1H, d, *J*=13.4Hz), 2.51 (1H, dd, *J*=18.3, 1.8Hz), 2.62 (1H, dd, *J*=16.2, 1.8Hz), 2.68 (1H, m), 3.14 (1H, dd, *J*=18.3, 1.8Hz), 3.16 (1H, m), 4.39 (1H, dd, *J*=6.4, 3.1Hz), 4.89 (1H, dd, *J*=3.1, 1.8Hz), 4.91 (1H, m), 5.04 (1H, m), 5.13 (1H, s), 5.15 (1H, m), 5.30 (1H, d, J=1.2Hz), 5.68 (1H, dd, *J*=3.1, 0.6Hz), 5.70 (1H, m), 6.09 (1H, dd, *J*=3.1, 0.6Hz), 6.10 (1H, m).

Compound 6: achromatic oily substance, ^{1}H-NMR (in methanol-d_4) δ : 1.34 (3H, d, *J*=7.0Hz), 1.98 (3H, d, *J*=0.9Hz), 2.15 (1H, dd, *J*=18.6, 0.9Hz), 2.58 (1H, dd, *J*=15.0, 3.7Hz), 2.75 (1H, dd, *J*=15.0, 4.3Hz), 2.81 (1H, dd, *J*=18.6, 6.7Hz), 3.50 (1H, m), 5.25 (1H, d, *J*=1.8Hz), 5.39 (1H, m), 5.51 (1H, dd, *J*=10.4Hz), 5.66 (1H, d, *J*=3.1Hz), 5.72 (1H, m), 5.78 (1H, d, *J*=1.8Hz), 6.17 (1H, d, *J*=0.9Hz), 6.20(1H, d, *J*=3.7Hz).

Compound 7: achromatic oily substance, ^{1}H-NMR (in methanol-d_4) δ : 1.15 (3H, d, *J*=6.4Hz), 1.89 (3H, s), 2.16 (1H, m), 2.28 (3H, s), 2.32 (1H, 16.2, 11.6Hz), 2.32 (1H, dd, *J*=13.7, 2.4Hz), 3.09 (1H, dd, *J*=13.7, 10.7Hz), 3.87 (1H, m), 4.19 (1H, d, *J*=10.4Hz), 5.01 (1H, ddd, *J*=10.7, 10.4, 2.4Hz), 5.57 (1H, d, *J*=3.1Hz), 5.66 (1H, s), 6.04 (1H, d, *J*=3.4Hz), 6.09 (1H, s).

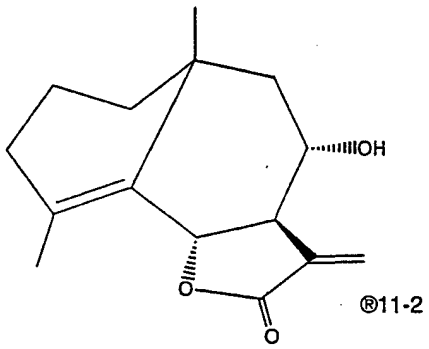
Compound 9: achromatic oily substance, ^{1}H-NMR (in chloroform-d_1) δ : 1.72 (3H,s), 2.01 (3H,s), 2.04 (1H,d, *J*=13.9 Hz), 2.46 (1H,d, *J*=10.7 Hz), 2.53 (1H,d, *J*=13.4 Hz), 2.68 (1H,ddd, *J*=15.8,1.5,1.5 Hz), 2.75 (1H,d, *J*=14.3 Hz), 2.75 (1H,d, *J*=14.3 Hz), 3.93 (1H,m), 4.24 (1H,dd, *J*=6.3,3.6 Hz), 4.35 (1H,d, *J*=3.7 Hz), 5.11 (1H,dt, *J*=11.3,3.1 Hz), 5.21 (1H,s), 5.38 (1H,brd.s), 5.73 (1H,brd.s), 5.79 (1H,dd, *J*=2.7,0.6 Hz), 6.20 (1H,s), 6.26 (1H,d. *J*=3Hz).

Compound 10: achromatic oily substance, ^{1}H-NMR (in chloroform-d_1) δ : 1.64(3H,s), 2.02 (1H,d,*J*=13.4 Hz), 2.02 (3H,s), 2.47 (1H,d,*J*=13.4 Hz), 2.54 (1H,d,*J*=13.4 Hz), 2.66 (1H, d,*J*=14.0 Hz), 2.66 (1H, d,*J*=14.0 Hz), 2.74 (1H,d,*J*=16.3 Hz), 3.14 (3H,s),

3.80 (1H,m), 4.18 (1H,dd,*J*=6.6,3.4 Hz), 4.40 (1H,d,*J*=3.4 Hz), 5.08 (1H,dt,*J*=11.6,3.1 Hz), 5.21 (1H,s), 5.39 (1H,s), 5.73 (1H,brd.s), 5.73 (1H,s), 6.21 (1H,d,*J*=0.9 Hz), 6.24 (1H,d,*J*=2.7 Hz).

Example 2. Production of the compound of formula 11-1

A material, Rothin-A shown in the following formula 11-2:



®11-2

can be obtained from *Artemisia* rothrockii, according to the method described in M. A. Irwin, T. A. Geissman, *Phytochemistry*, vol.10, p.637-645 (1971) or an equivalent method. That is to say, 0.78kg of crushed *Artemisia* rothrockii is extracted with 2.5L of chloroform 3 times, then the obtained extract is concentrated. 2L of methanol water (3:1) is added to the concentrated extract, which is then suspended and extracted with 1L of hexane. The obtained water layer is concentrated to 0.3L under reduced pressure, and extracted with 0.5L of chloroform 4 times. The residue obtained by concentrating such obtained extract is purified with silica gel column chromatography. The inner diameter of the used column is 8cm, and the height is 37cm. As elution solvents, first a mixed solvent of chloroform and benzene is used, then the polarity of the solvent is gradually increased by adding acetone. 0.5L per fraction is separated, and a total of 30 fractions are separated. The 6^{th} to 10^{th} fractions are together concentrated to obtain 0.26g of Rothin-A.

The obtained Rothin-A is induced to the compound of formula 11-1 according to the following method: in a 20mL of reaction vessel, 100mg (0.4mM) of Rothin-A is dissolved into 1mL of dried pyridine, and while the solution is stirred with multi-shaft stirring blades, it is cooled down to 10°C. Then, 50.1mg (0.19mM) of metacryloyl chloride is slowly added to the solution, followed by stirring for 1 to 3 hours. After completing the reaction, 20mL of water is added to the reaction solution and extracted with low polar organic solvent, chloroform. The organic layer is washed and dried with anhydrous sodium sulfate, and the solvent was removed under reduced pressure to obtain the compound of interest shown in formula 11-1.

Example 3. Production of the compound of formula 8

Process 3-a Iodination of the compound of formula 8-2

4 mol of triphenyl phosphine and 2 mol of triiodoimidazole are used for 1 mol of the compound of formula 8-2. The compound of formula 8-2, triphenyl phosphine and triiodoimidazole are put into a reaction vessel and dissolved into toluene. The reaction solution is stirred with multi-shaft stirring blades. Then, the reaction solution was heated up to 120°C. This reaction is completed within 30 minutes to 30 hours. After completing the reaction, the reaction solution is washed and the solvent is removed under reduced pressure, to obtain the crude crystal of the compound of interest.

Process 3-b Conversion into the compound of formula 8

1 mol of azobisisobutyronitrile and 2 mol of tributylstannane are used for 1 mol of the compound obtained by process 3-a. The above-stated compound and azobisisobutyronitrile are put into a reaction vessel and dissolved into the mixed solvent of benzene and methanol. Then, tributylstannane is dropped thereinto and the reaction solution is heated under reflux. The reaction time is approx. 1 to 10 hours. After completing the reaction, the solvent is removed under reduced pressure. The residue is purified with silica gel column chromatography using the mixed solvent of n-hexane

and ethyl acetate as a developing solvent, to obtain the compound of formula 8.

Production of a material, the compound of formula 8-2

Using a Soxhlet extractor, 3kg of dried composite plant, *Eremanthus* goyanensis is extracted with chloroform for 2 days. After the solvent is removed under reduced pressure, the extract is concentrated, and the residue is dissolved into 250mL of ethanol. Then, 250mL of hot water containing 10g of lead acetate and 3mL of acetic acid is added thereto to dilute the solution. After the solution is left for 2 days, the supernatant liquid is filtrated and the solvent is removed under reduced pressure to concentrate the solution. By extracting the residue with chloroform and concentrating again, an oily product is obtained. This oily product is crystallized from benzene to obtain the compound of formula 8-2, goyazensolide.

Example 4. Production of the compound of formula 8-1

0.5g of Adam's platinum oxide catalyst is used for 1 mol of the compound of formula 8. The compound of formula 8 is put into a reaction vessel and dissolved into ethanol. Adam's platinum oxide is added thereto and shaken strongly under hydrogen ambient at room temperature. The reaction time is 0.5 to 5 hours. After completing the reaction, the catalyst is filtrated and the solvent is removed from the filtrate under reduced pressure. The obtained residue is purified with silica gel column chromatography, using the mixed solvent of n-hexane and ethyl acetate as a developing solvent, to obtain the compound of formula 8-1.

Example 5. Production of methoxyl derivative of the compound of formula 1

1.5 mol of iodomethane is used for 1 mol of the compound of formula 1. Acetonitrile is used as a reaction solvent. A reflux condenser is installed in a reaction vessel. The mixture of the above-stated compositions is put into the reaction vessel, then 1.5 mol of silver oxide is further added thereto. The mixure is heated under reflux, while it is stirred with multi-shaft stirring blades. The reaction time is approx.

1 to 24 hours. After completing the reaction, the reaction solution is filtrated and the filtrate is concentrated under reduced pressure. The obtained residue is purified with silica gel column chromatography, using the mixed solvent of chloroform and methanol as an elution solvent, thereby obtaining methoxyl derivative of the compound of formula 1.

Example 6. Production of acetoxyl derivative of the compound of formula 1

1.5 mol of acetic anhydride is used for 1 mol of the compound of formula 1. Pyridine is used as a reaction solvent. The mixture of the above-stated compositions put into the reaction vessel is stirred with multi-shaft stirring blades. The reaction time is approx. 1 to 3 hours. After completing the reaction, water is added thereto and left for 30 minutes. The reaction solution is extracted with a lower polar organic solvent and chloroform. Then, the organic layer is washed. The solvent of the organic layer is removed to obtain the acetoxyl derivative of interest.

Example 7. Production of triethylsilyloxyl derivative of the compound of formula 1

1.2 mol of triethylsilyl chloride is used for 1 mol of the compound of formula 1. As reaction solvents, dimethylformamide anhydride and 2.5 mol of imidazole are used. First, dimethylformamide is added to a reaction vessel, and then the compound of formula 9 is dissolved thereto. The solution is cooled down to 0°C, and triethylsilyl chloride and imidazole are slowly added thereto. The mixture of the above-stated compositions is stirred with multi-shaft stirring blades. The reaction time is approx. 0.5 to 3 hours. After completing the reaction, the reaction solution is directly purified with silica gel column chromatography. As an elution solvent, a mixed solvent of chloroform and ethyl acetate is used. According to this process, triethylsilyloxyl derivative of the compound of formula 1 of interest is obtained.

Example 8. Production of acetal compound of the compound of formula 1

10 mol of ethylene glycol, and 0.01 mol of para-toluene sulfonic acid

monohydrate are used for 1 mol of the compound of formula 1. As a reaction solvent, benzene is used. After a reflux condenser with Dean-Stark water separator is installed, the mixture of these solutions is heated under reflux. The reaction time is approx. 10 to 20 hours. After completing the reaction, the solution is cooled down and washed with saturated sodium bicarbonate water, then with water. The solvent of the organic layer is removed to obtain the acetal compound of interest.

Example 9. Production of hydrazone derivative of the compound of formula 1

2 mol of N,N-dimethylhydrazine is used for 1 mol of the compound of formula 1. As a reaction solvent, a mixture of ethanol and acetic acid is used. The reaction mixture is heated under reflux in a reaction flask in which a reflux condenser is installed. The reaction time depends on conditions, but it is approx. 10 to 20 hours. After completing the reaction, the reaction solution is cooled down, and water and a low polar organic solvent, chloroform are added thereto. Then, the reaction solution is washed with 3% hydrochloric acid solution, then with water. The solvent of the organic layer is removed to obtain the N,N-dimethylhydrazine compound of interest.

Example 10. Production of oxime derivative of the compound of formula 1

5 mol of hydroxylamine hydrochloride and 1.1 mol of diazabicyclo octane are used for 1mol of the compound of formula 1. As a solvent, methanol is preferable. The compound of the above formula 1, the reagent and the solvent are put into a reaction flask, and the reaction solution is fully stirred with multi-shaft stirring blades in order to stimulate the reaction. The reaction is carried out at room temperature. The reaction is completed in 1 to 2 days. After completing the reaction, the solvent is removed and water is added. Then, the reaction solution is controlled to pH 4 by adding concentrated hydrochloric acid, and extracted with a low polar organic solvent, chloroform. The solvent of the organic layer is removed to obtain the oxime compound of interest.

Example 11. Production of the compound of formula 13

1.2 mol of acetic anhydride is used for 1 mol of the compound of formula 1. The compound of formula 1 is put into a reaction vessel and dissolved into pyridine. After adding thereto acetic anhydride, the solution is stirred at room temperature. The reaction time is approx. 1 to 24 hours. After the reaction, the reaction solution is put into ice water and then the eluted crystal is filtrated, thereby the compound of formula 13 of interest is obtained.

Example 12. Production of the compound of formula 14

1.2 mol of normal decanoyl chloride is used for 1 mol of the compound of formula 1. The compound of formula 1 is put into a reaction vessel and dissolved in pyridine. After adding normal decanoyl chloride, the mixture is stirred at room temperature. The reaction time is approx. 1 to 24 hours. After completing the reaction, water is added to the reaction solution, and then the solution is extracted with ethyl acetate. The solvent is removed from the layer of ethyl acetate under reduced pressure, and the residue is purified with silica gel column chromatography. The mixed solvent of ethyl acetate and hexane is used as an elution solvent. According to the above process, the compound of formula 14 of interest is obtained.

Example 13. Production of the compound of formula 15

The compound of formula 1 is put into a reaction vessel and dissolved in acetone. While stirring the reaction solution at room temperature, Jones reagent is dribbled thereinto drop by drop. The dropping is finished when the reaction solution keeps red color. The reaction solution is poured to ice water, and extracted with dichloromethane. The solvent is removed under reduced pressure, and the obtained residue is purified with silica gel column chromatography. The mixed solvent of ethyl acetate and hexane is used as an elution solvent. According to the above process, the compound of formula 15 of interest is obtained.

Example 14. Production of the compounds of formulas 16, 17 and 18

20ml of 0.5% sulfuric acid/acetone is used for 100mg of the compound of formula 15. The compound of formula 15 is put into a reaction vessel and dissolved in 4ml of 0.5% sulfuric acid/acetone. The reaction solution is stirred at room temperature. The reaction time is approx. 1 to 5 hours. After completing the reaction, the solvent is removed from the reaction solution under reduced pressure. Water is added to the residue, and extracted with chloroform. The organic layer is washed with sodium bicarbonate water, and the solvent is removed under reduced pressure. The residue is purified with silica gel column chromatography, thereby obtaining the compounds of formulas 16, 17 and 18 of interest.

Both physical properties and ^{1}H-NMR chemical shift values of compounds13 to 18 are shown below. Regarding the symbols used, s denotes singlet, d denotes doublet, dd denotes double doublet, ddd denotes double double doublet, t denotes triplet, m denotes multiplet, j (italic) denotes coupling constant, and Hz denotes herz, respectively.

Compound 13: achromatic crystal, ^{1}H-NMR(in chloroform-d_1) δ : 6.49 (1H,s), 6.29 (1H,s), 6.27 (1H,d, J=2 Hz), 6.07 (1H,s), 5.97 (1H,s), 5.71 (1H,s), 5.60 (1H,s), 5.13 (1H,ddd, J=12, 11, 4 Hz), 4.28 (1H,d, J=3 Hz), 3.35 (m), 2.47 (1H,dd, J=12, 12 Hz), 2.70 (1H,dd, J=12, 4 Hz), 2.02 (3H,s), 1.92 (3H,s), 1.88 (3H,s), 1.74 (3H,s)

Compound 14: achromatic oily substance, ^{1}H-NMR(in chloroform-d_1) δ : 6.59 (1H,s), 6.36 (1H,s), 6.33 (1H,d, J=2 Hz), 6.14 (1H,s), 6.04 (1H,s), 5.77 (1H,d, J=2 Hz), 5.67 (1H,s), 5.20 (1H,ddd, J=11, 11, 4 Hz), 4.35 (1H,d, J=3 Hz), 3.42 (m), 2.77 (1H,dd, J=12, 4 Hz), 2.54 (1H,dd, J=12, 11 Hz), 1.98 (3H,s), 1.95 (3H,s), 1.81 (3H,s)

Compound 15: achromatic crystal, ^{1}H-NMR(in chloroform-d_1) δ : 6.43 (d, J=3 Hz), 6.19 (1H,s), 6.04 (1H,q, J=1 Hz), 5.96 (1H,d, J=3 Hz), 5.94 (1H,s), 5.73 (1H,s), 5.28 (1H,td, J=11, 4 Hz), 4.78 (1H,d, J=5 Hz), 3.96 (1H,dddd, J=11, 5, 3, 3 Hz), 2.73 (1H,dd, J=12, 4 Hz), 2.39 (1H,t, J=12 Hz), 2.21 (3H,d, J=1 Hz), 1.93 (3H,s)

Compound 16: achromatic amorphous substance, ^{1}H-NMR(in chloroform-d_1) δ : 6.21 (1H,s), 6.21 (1H,d, J=3 Hz), 6.20 (1H,s), 5.72 (1H,s), 5.64 (1H,d, J=3 Hz), 5.00 (1H,ddd, J=10, 12, 2 Hz), 4.10 (1H,dddd, J=10, 10, 3, 3 Hz), 4.00 (1H,d, J=10 Hz), 3.27 (1H,dd, J=12, 12 Hz), 2.37 (1H,dd, J=12, 2 Hz), 2.46 (3H,s), 2.32 (3H,s), 2.00 (3H,s)

Compound 17: achromatic amorphous substance, ^{1}H-NMR(in chloroform-d_1) δ : 6.29 (1H,d, J=3Hz), 6.21 (1H,s), 6.08 (1H,s), 5.72 (1H,d, J=3 Hz), 5.70 (1H,s), 5.22 (1H,s), 5.20 (1H,s), 5.11 (1H,ddd, J=10, 5, 3 Hz), 4.20 (1H,d, J=9 Hz), 3.78 (1H,dddd, J=10, 9, 3, 3 Hz), 3.45 (1H,s), 2.54 (1H,dd, J=15, 3 Hz), 2.36 (1H,dd, J=15, 5 Hz), 2.31 (3H,s), 2.00 (3H,s)

Compound 18: achromatic amorphous substance, ^{1}H-NMR(in chloroform-d_1) δ : 6.29 (1H,d, J=3 Hz), 6.18 (1H,s), 6.05 (1H,s), 5.72 (1H,s), 5.71 (1H,s), 5.22 (1H,ddd, J=10, 7, 4 Hz), 4.21 (1H,d, J=9 Hz), 4.02 (1H,m), 2.89 (1H,s), 2.32 (1H,s), 2.26 (1H,dd, J=15, 4 Hz), 2.03 (1H,dd, J=15, 7 Hz), 2.01 (3H,s), 1.41 (3H,s)

Test Example 1. Anti-leishmanial activity test

Anti-leishmanial activity was tested according to the following method:

Test Method

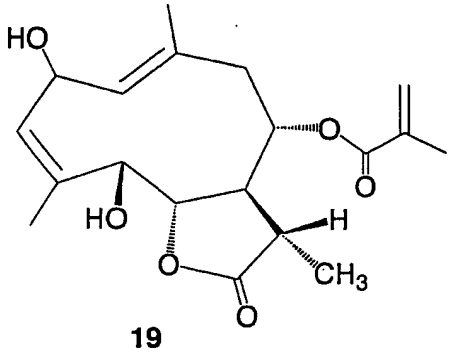
1. The method of culturing a protozoan, Leishmania

Leishmania major was cultured on Medium 199 in a tissue culture flask (25cm^2 size) placed in a CO_2 incubator, wherein the conditions were kept at 26.5°C and 5% CO_2. It was subcultured by diluting to 50 to 100 times at intervals of one or two days. Protozoans used for assay were counted on a blood cell counting chamber just before the use, and then these were diluted with Medium 199 to 1×10^6/ml.

2. The method of evaluating anti-leishmanial activity

The sample dissolved in DMSO was diluted to 50 times on Medium 199, and then the sample solution was sterilized with a membrane filter (0.2 μm). 9 types of sample solution, the concentration of each of which was different, was prepared by doubling dilution. Both 50 μl of each sample solution each having a different

concentration and 50 μL of leishmania whose concentration was controlled were put onto a microtiter plate to prepare total 100 μl of culture liquid. After the incubation at 26.5°C at 5% CO_2 for 24 hours, the number of protozoans in each well was counted on a blood cell counting chamber. Regarding this test, 3 wells were used for each sample and each concentration, and then a graph was made on the basis of the obtained mean values and mean errors to measure IC_{50} (50% inhibitory concentration). Furthermore, a sample containing only medium instead of sample solution was used as a control, whose concentration was set to 0. The results are shown in Table 1. The compounds shown in the following formulas 19 to 24 were used as control examples. The compounds described in the column of compounds correspond to the ones shown in the above formulas 1 to 7, 9, 10 and 13 to 18.



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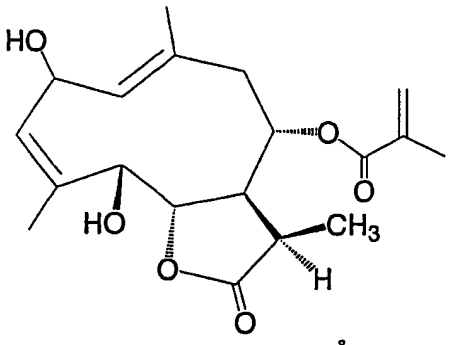

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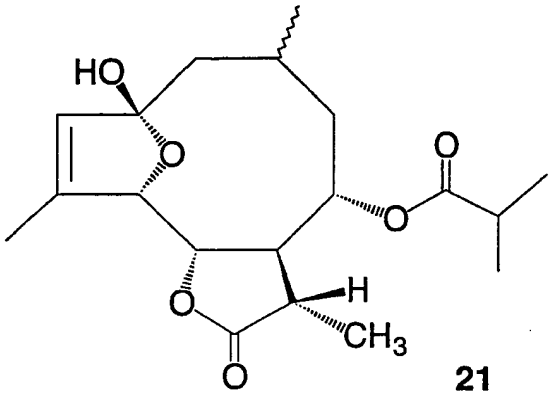

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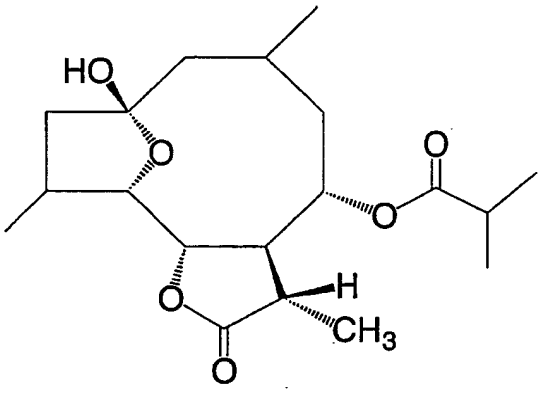

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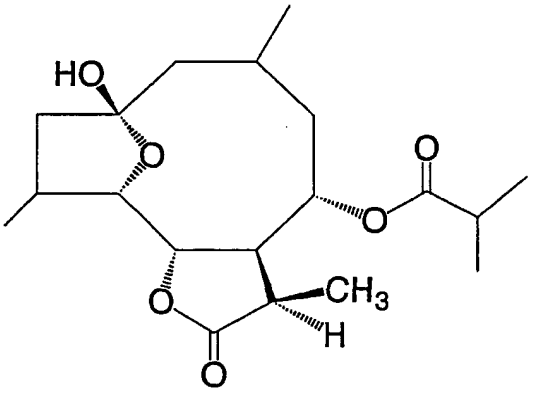

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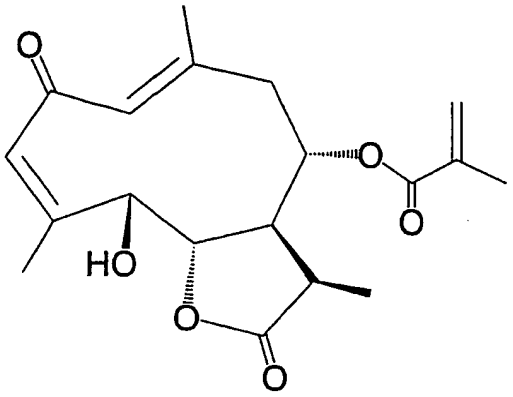

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Table 1. Results of anti-leishmanial activity test

Test Examples	Compounds	IC_{50} (μ g/ml)
1	2	<0.1
2	13	<0.1
3	15	<0.1
4	16	<0.1
5	17	<0.1
6	4	0.1
7	18	0.1
8	1	0.2
9	6	0.5
10	3	0.6
11	5	0.6
12	14	0.8
13	9	1.1
14	10	1.1
15	7	1.5
Control Example 1	20	7.4
Control Example 2	24	12.0
Control Example 3	19	26.0
Control Example 4	21	35.0
Control Example 5	22	71.0
Control Example 6	23	79.0
Control Example 7	Amphotericin B	<0.1
Control Example 8	Pentamidine	4.1
Control Example 9	Sb (III)*	<0.1
Control Example 10	Sb (V) **	10.4

* dipotassium bistartrate antimonate

**antimony pentachloride

As is clear from this table, any of the compounds of the present invention has activity equivalent to or greater than previously known antiprotozoal agents. The compounds of formulas 19 to 24, which are similar to the compounds of the present invention, but do not satisfy the basic formula of the present invention, do not have anti-leishmanial activity.

Acute toxicity test of methanol extract of *Elephantopus* mollis H.B.K.

An acute toxicity test using mice was carried out for the extract that was

obtained by extracting with methanol, *Elephantopus* mollis H.B.K. containing the compounds of formulas 1 to 7, 9 and 10 of the present invention. That is to say, the extract was suspended in corn oil, and then 280mg/kg, 240mg/kg, 200mg/kg, 160mg/kg, 120mg/kg, 80mg/kg and 40mg/kg of the extract was intraperitonealy administered to each mouse. All of these mice were survived after 7 days of observation. 50% lethal dose of a control agent, amphotericin B is 88mg/kg. Furthermore, in respect of the mice to which 280mg/kg and 240mg/kg were administered, when compounds in blood were detected with a high performance liquid chromatography, the obtained chromatogram was similar to that of methanol extract, and so it was confirmed that these compounds had moved into the blood. From these results, it is considered that the compounds of formulas 1 to 7, 9 and 10 does not have any serious toxicity problems, and that the pharmaceutical use of these compounds as an extract poses no problems either.

Pharmaceutical formulation example 1.

According to standard techniques, the compound shown in formula 1 was mixed with lactose, corn starch and carboxy methylcellulose calcium. Then, according to standard techniques, kneaded liquid prepared with methylcellulose and purified water was kneaded in. After that, the mixture was dried to obtain granules. Magnesium stearate was further added to the granules and mixed, and then tablets were produced by compression molding.

The compound of formula 1	100mg
Lactose	33mg
Corn starch	16mg
Carboxy methylcellulose calcium	12mg
Methylcellulose	6mg
Magnesium stearate	2mg
Total Amount	169mg

Pharmaceutical formulation example 2.

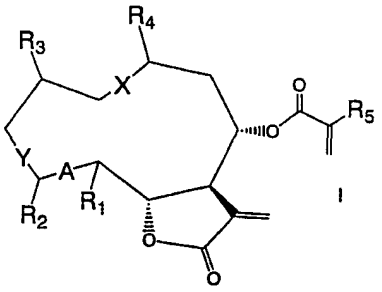
The compound of formula 1 was dissolved in physiological salt solution containing 30% (w/v) polyethylene glycol 400 to prepare 0.05% solution of the compound. Then, according to standard techniques, the solution was sterilized and filtrated, and 50 ml of the solution was independently poured into each vial, so that 10mg of the compound could be contained in each vial. Thus, intravenous agents were obtained.

Industrial Applicability of the Invention

The present invention provides a pharmaceutically useful sesquiterpenoic compound that has antiprotozoal activity, particularly anti-leishmanial activity, and pharmaceutical formulations comprising the same, particularly an antiprotozoal agent.

CLAIMS

1. A sesquiterpenoic compound shown in the following formula I, IIa, IIIb or IV, or a derivative thereof :



I

wherein

-X-, -Y- and –A- represent a single bond or a double bond,

R_1 is H, OH, =O, an -O- bond to C at position 2 or 4, or a single bond to C at position 10,

R_2 is methyl or methylene,

R_3 is H, OH or =O,

R_4 is methyl or methylene,

R_5 is methyl, and

R_3 and R_4 may together form –O-CO-;

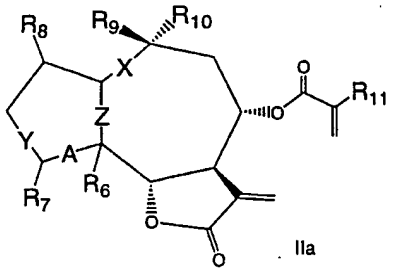
with the exception that the following compounds (1) to (4) are excluded from the compounds shown in said formula I or derivatives thereof:

(1) a compound wherein –X- and –Y- represent a double bond, -A- represents a single bond, each of R_2, R_4 and R_5 is methyl, R_3 is =O, R_1 is OH, acetyloxy, =O, or p-Br-benzenesulfonyloxy;

(2) a compound wherein –X- represents a double bond, -Y- and –A- represent a single bond, R_1 is an –O- bond to C at position 4, R_2 and R_5 are methyl, R_3 and R_4 are respectively OH and methyl, or R_3 and R_4 together form –O-C(O)- or a methylacetal or ethylacetal derivative thereof;

(3) a compound wherein –X- and –A- represent a double bond, -Y- represents a single bond, R_1 is H, R_2 and R_5 are methyl, R_3 and R_4 together form –O-C(O)-; and

(4) a compound wherein –X- and –Y- represent a double bond, -A- represents a single bond, R_1 represents a –O-bond to C at position 2, R_2, R_4 and R_5 are methyl, R_3 is OH, methoxyl or ethoxyl;



IIa

wherein

-X-, -Y-, -Z- and –A- represent a single bond or a double bond,

R_6 does not exist or represents OH,

R_7 is methyl or methylene,

R_8 is OH or =O,

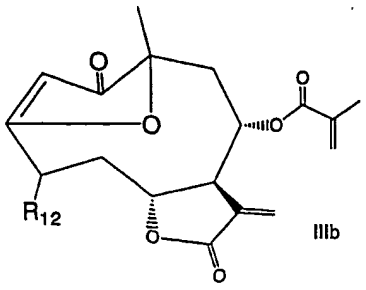
R_9 and R_{10} do not exist or represent methyl, methylene or OH, and

R_{11} represents methyl;

with the exception that the following compounds (1) and (2) are excluded from the compounds shown in said formula IIa or derivatives thereof:

(1) a compound wherein each of –X-, -Z- and –A- represents a single bond, -Y- represents a double bond, R_6 is OH, each of R_7, R_{10} and R_{11} is methyl, R_8 is =O, and R_9 is OH;

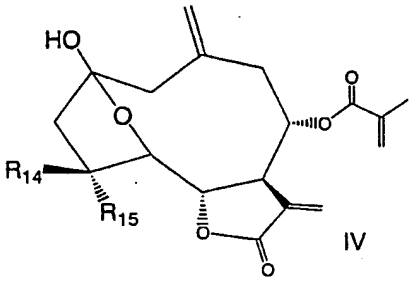
(2) a compound wherein -A- and –Z- represent a single bond, -X- and –Y- are a double bond, R_6 is OH, R_7 and R_{11} are methyl, R_8 is =O, and R_9 and R_{10} together form methyl;



IIIb

wherein

R_{12} is methyl or methylene; or

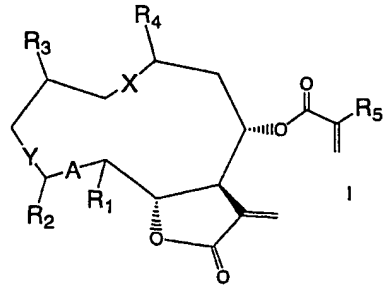


IV

wherein

R_{14} and R_{15} respectively represent methyl or OH, and alkoxyl.

2. A pharmaceutical formulation comprising a sesquiterpenoic compound shown in the following formula I, IIa, IIIc or IV, or a derivative thereof:



I

wherein

-X-, -Y- and –A- represent a single bond or a double bond,

R_1 is H, OH, =O, an -O- bond to C at position 2 or 4, or a single bond to C at position 10,

R_2 is methyl or methylene,

R_3 is H, OH or =O,

R_4 is methyl or methylene,

R_5 is methyl, and

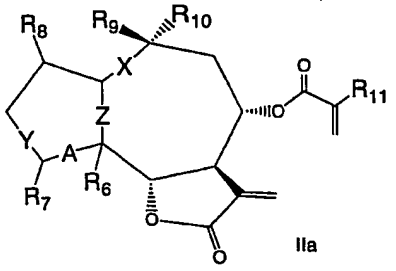
R_3 and R_4 may together form –O-CO-;

with the exception that the following compounds (1) to (3) are excluded from the compounds shown in said formula I or derivatives thereof:

(1) a compound wherein –X- and –Y- represent a double bond, -A- represents a single bond, each of R_2, R_4 and R_5 is methyl, R_3 is =O, R_1 is OH, acetyloxy, =O or p-Br-benzenesulfonyloxy;

(2) a compound wherein –X- represents a double bond, -Y- and –A- represent a single bond, R_1 is an –O- bond to C at position 4, R_2 and R_5 are methyl, R_3 and R_4 are respectively OH and methyl, or R_3 and R_4 together form –O-C(O)- or a methylacetal or ethylacetal derivative thereof;

(3) a compound wherein –X- and –A- represent a double bond, -Y- represents a single bond, R_1 is H, R_2 and R_5 are methyl, R_3 and R_4 together form –O-C(O)-;



IIa

wherein

-X-, -Y-, -Z- and –A- represent a single bond or a double bond,

R_6 does not exist or represents OH,

R_7 is methyl or methylene,

R_8 is OH or =O,

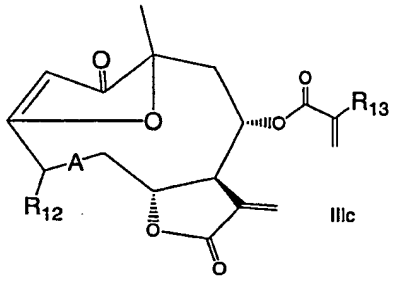
R_9 and R_{10} do not exist or represent methyl, methylene or OH, and

R_{11} represents methyl;

with the exception that the following compounds (1) and (2) are excluded from the compounds shown in said formula IIa or derivatives thereof:

(1) a compound wherein each of –X-, -Z- and –A- represents a single bond, -Y- represents a double bond, R_6 is OH, each of R_7, R_{10} and R_{11} is methyl, R_8 is =O, and R_9 is OH;

(2) a compound wherein -A- and –Z- represent a single bond, -X- and –Y- represent a double bond, R_6 is OH, R_7 and R_{11} are methyl, R_8 is =O, and R_9 and R_{10} together form methyl;



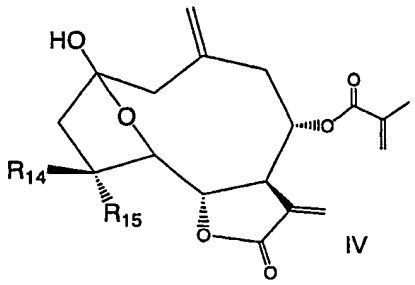
IIIc

wherein

-A- represents a single bond or a double bond,

R_{12} is methyl or methylene, and

R_{13} is methyl; or

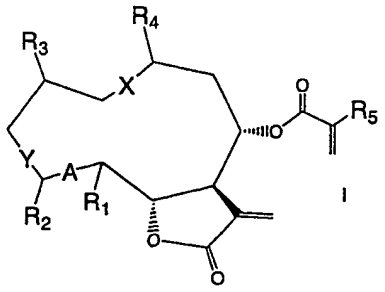


IV

wherein

R_{14} and R_{15} respectively represent methyl or OH, and alkoxyl.

3. An antiprotozoal agent comprising a sesquiterpenoic compound shown in the following formula I, IIa, IIIc and IV, or a derivative thereof:



I

wherein

-X-, -Y- and –A- represent a single bond or a double bond,

R_1 is H, OH, =O, an -O- bond to C at position 2 or 4, or a single bond to C at position 10,

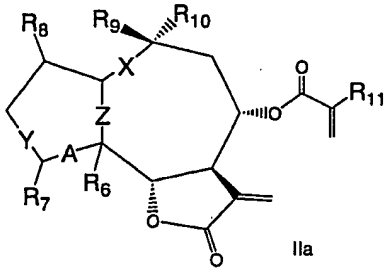
R_2 is methyl or methylene,

R_3 is H, OH or =O,

R_4 is methyl or methylene,

R_5 is methyl, and

R_3 and R_4 may together form –O-CO-;



IIa

wherein

-X-, -Y-, -Z- and –A- represent a single bond or a double bond,

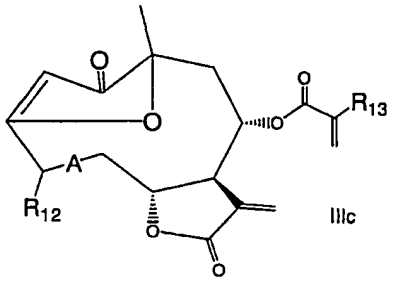
R_6 does not exist or represents OH,

R_7 is methyl or methylene,

R_8 is OH or =O,

R_9 and R_{10} do not exist or represent methyl, methylene or OH, and

R_{11} represents methyl;



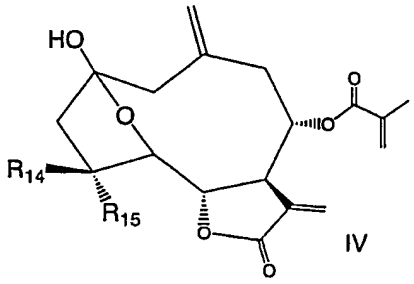
IIIc

wherein

-A- represents a single bond or a double bond,

R_{12} is methyl or methylene, and

R_{13} is methyl; or

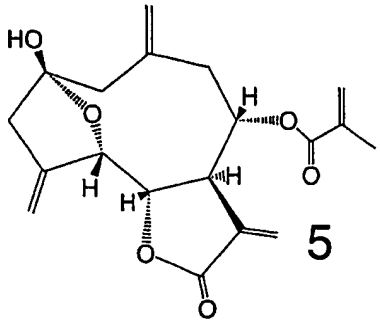


IV

wherein

R_{14} and R_{15} respectively represent methyl or OH, and alkoxyl.

4. A sesquiterpenoic compound shown in the following formula 5, 6, 7, 9, 10, 14 or 17, or a derivative thereof :



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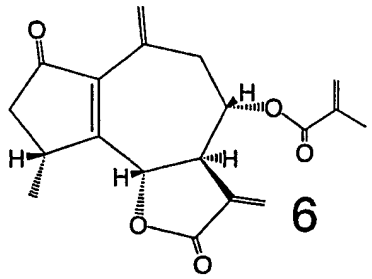

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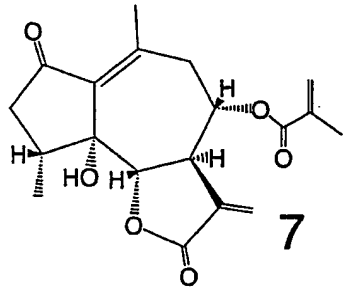

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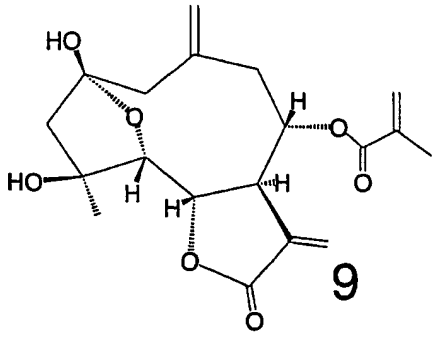

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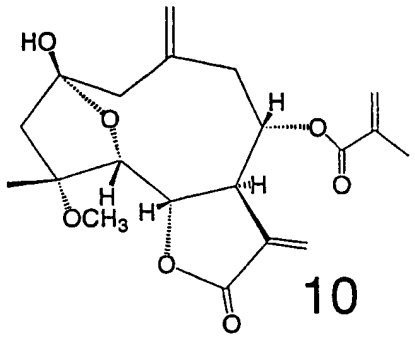

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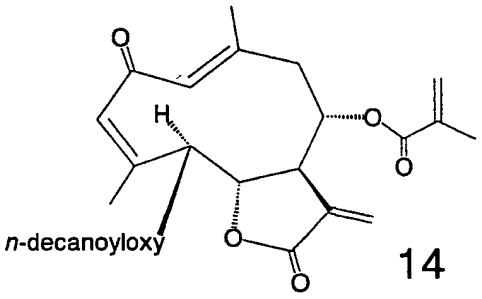

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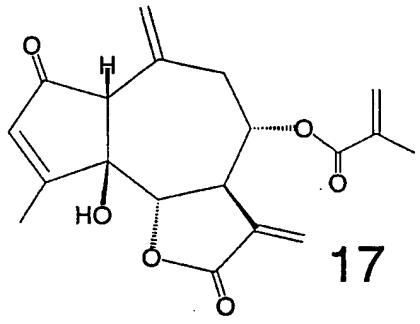
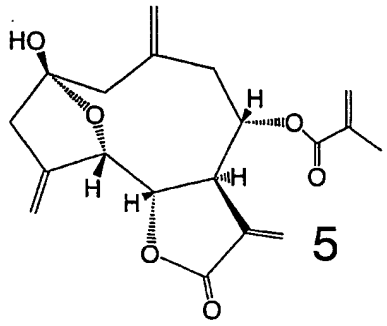


17

5. A pharmaceutical formulation comprising a sesquiterpenoic compound shown in the following formula 5, 6, 7, 9, 10, 14 or 17, or a derivative thereof:



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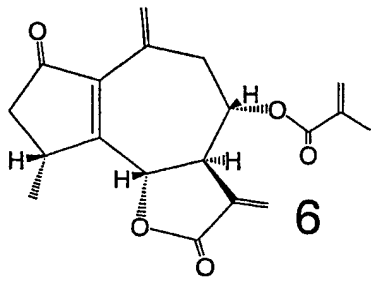

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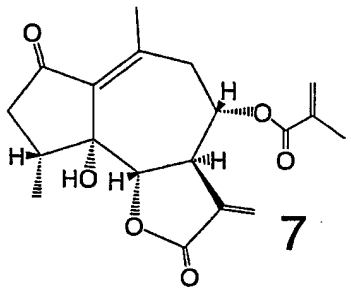

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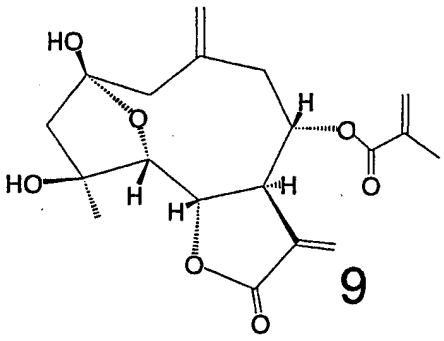

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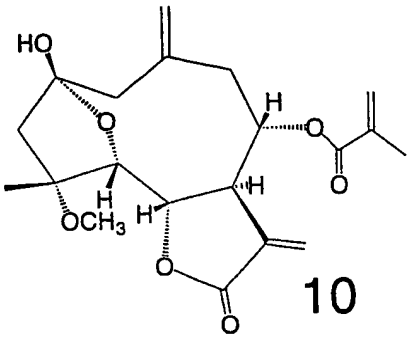

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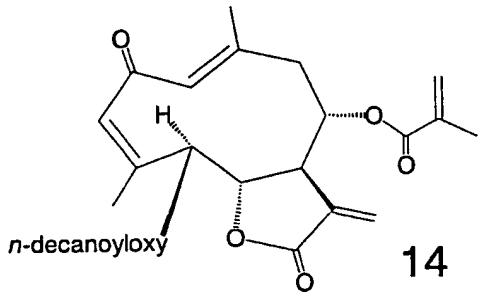

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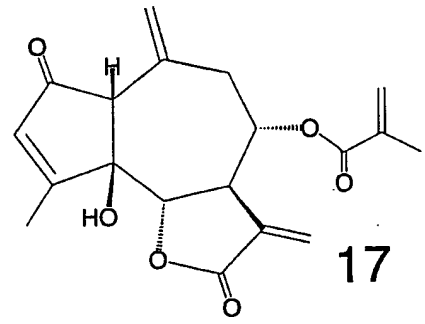
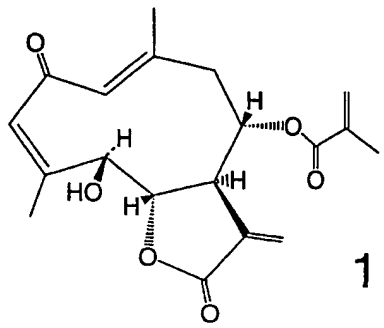


17

6. An antiprotozoal agent comprising a sesquiterpenoic compound shown in any one of the following formulas 1 to 7, 9, 10, 13 to 18 or a derivative thereof:



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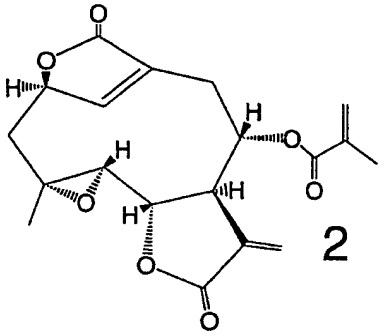

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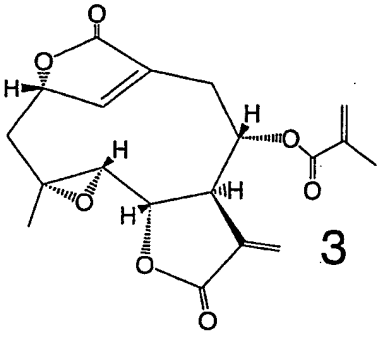

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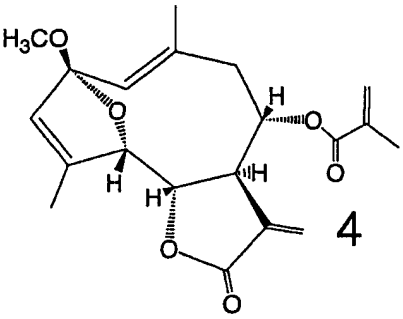

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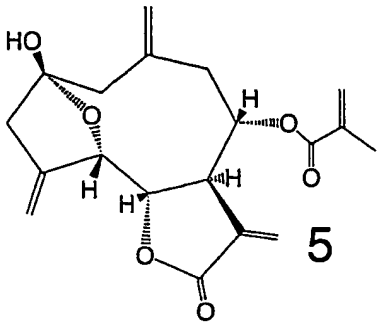

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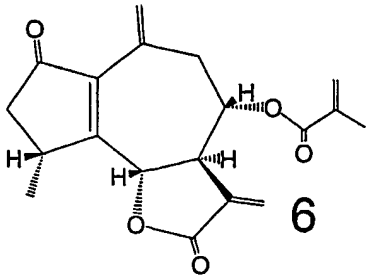

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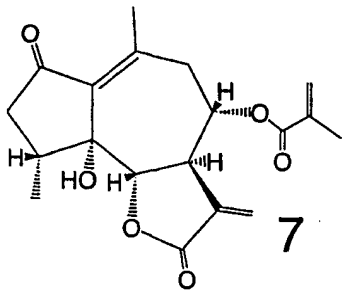

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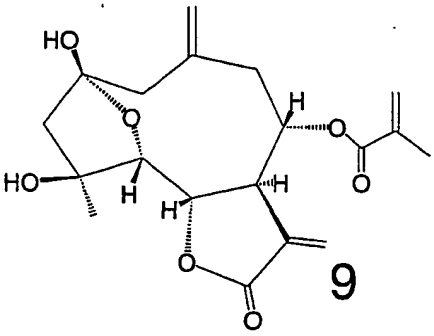

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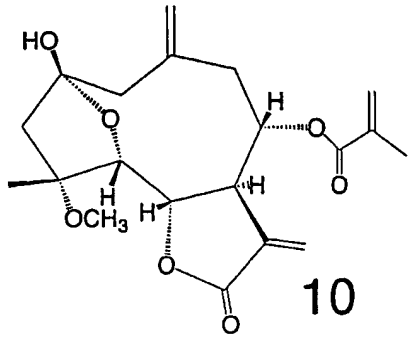
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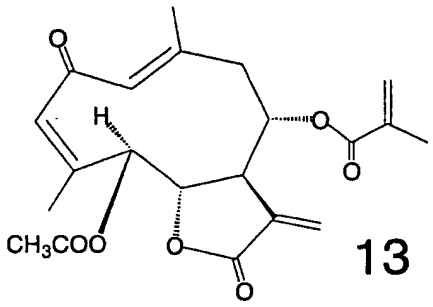
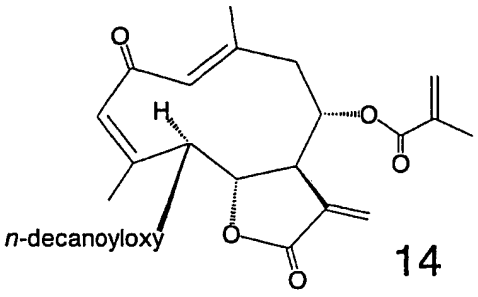
O
H
O
O
H
H
OCH3
H
O
O

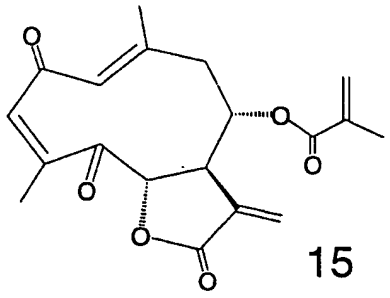
10


O
H
O
O
CH3COO
O
O

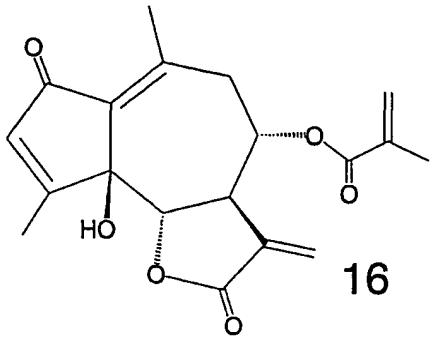
13


O
H
O
O
n-decanoyloxy
O
O

14


O
O
O
O
O
O

15



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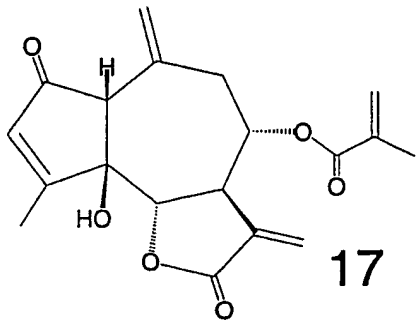

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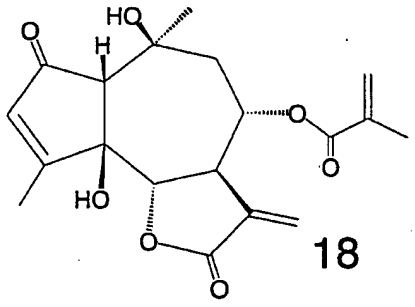
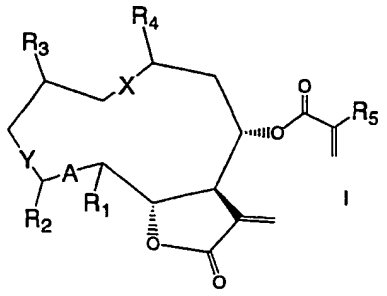


18

7. A pharmaceutical formulation which consists of a composition containing, as active ingredients, at least more than one compound selected from the group comprising sesquiterpenoic compounds of the following formula I IIa, IIIc and IV, and derivatives thereof, extracted from composite plants:



I

wherein

-X-, -Y- and –A- represent a single bond or a double bond,

R_1 is H, OH, =O, an -O- bond to C at position 2 or 4, or a single bond to C at position 10,

R_2 is methyl or methylene,

R_3 is H, OH or =O,

R_4 is methyl or methylene,

R_5 is methyl, and

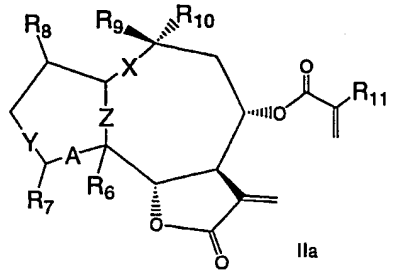
R_3 and R_4 may together form –O-CO-;

with the exception that the following compounds (1) to (3) are excluded from the compounds shown in said formula I or derivatives thereof:

(1) a compound wherein –X- and –Y- represent a double bond, -A- represents a single bond, each of R_2, R_4 and R_5 is methyl, R_3 is =O, R_1 is OH, acetyloxy, =O, or p-Br-benzenesulfonyloxy;

(2) a compound wherein –X- represents a double bond, -Y- and –A- represent a single bond, R_1 is an –O- bond to C at position 4, R_2 and R_5 are methyl, R_3 and R_4 are respectively OH and methyl, or R_3 and R_4 together form –O-C(O)- or a methylacetal or ethylacetal derivative thereof;

(3) a compound wherein –X- and –A- represent a double bond, -Y- represents a single bond, R_1 is H, R_2 and R_5 are methyl, R_3 and R_4 together form –O-C(O)-;



IIa

wherein

-X-, -Y-, -Z- and –A- represent a single bond or a double bond,

R_6 does not exist or represents OH,

R_7 is methyl or methylene,

R_8 is OH or =O,

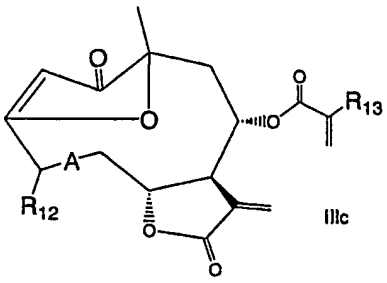
R_9 and R_{10} do not exist or represent methyl, methylene or OH, and

R_{11} represents methyl;

with the exception that the following compounds (1) and (2) are excluded from the compounds shown in said formula IIa or derivatives thereof:

(1) a compound wherein each of –X-, -Z- and –A- represents a single bond, -Y- represents a double bond, R_6 is OH, each of R_7, R_{10} and R_{11} is methyl, R_8 is =O, and R_9 is OH;

(2) a compound wherein -A- and –Z- represent a single bond, -X- and –Y- are a double bond, R_6 is OH, R_7 and R_{11} are methyl, R_8 is =O, and R_9 and R_{10} together form methyl;



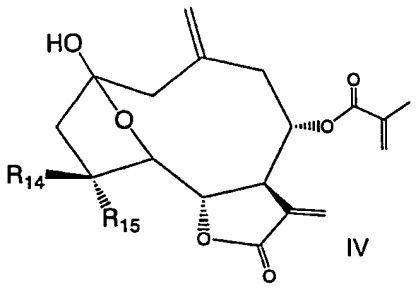
IIIc

wherein

-A- is a single bond or a double bond,

R_{12} is methyl or methylene, and

R_{13} is methyl; or

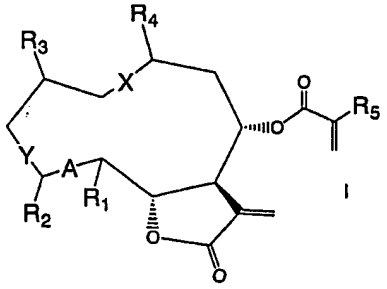


IV

wherein

R_{14} and R_{15} respectively represent methyl or OH, and alkoxyl.

8. An antiprotozoal agent which consists of a composition containing, as active ingredients, a sesquiterpenoic compound of the following formula I IIa, IIIc or IV, or a derivative thereof, extracted from a composite plant:



I

wherein

-X-, -Y- and –A- represent a single bond or a double bond,

R_1 is H, OH, =O, an -O- bond to C at position 2 or 4, or a single bond to C at position 10,

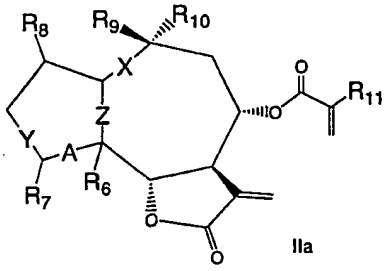
R_2 is methyl or methylene,

R_3 is H, OH or =O,

R_4 is methyl or methylene,

R_5 is methyl, and

R_3 and R_4 may together form –O-CO-;



IIa

wherein

-X-, -Y-, -Z- and –A- represent a single bond or a double bond,

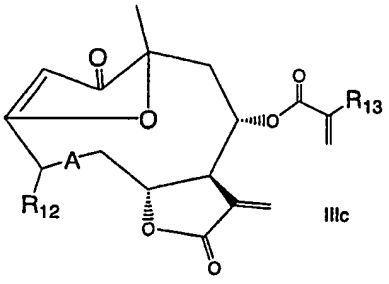
R_6 does not exist or represents OH,

R_7 is methyl or methylene,

R_8 is OH or =O,

R_9 and R_{10} do not exist or represent methyl, methylene or OH, and

R_{11} represents methyl;



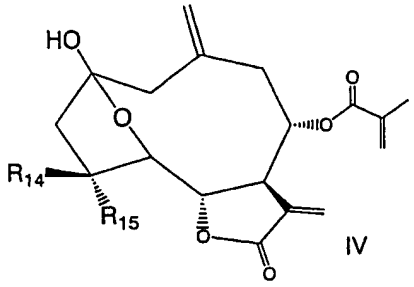
IIIc

wherein

-A- is a single bond or a double bond,

R_{12} is methyl or methylene, and

R_{13} is methyl; or



IV

wherein

R_{14} and R_{15} respectively represent methyl or OH, and alkoxyl.

INTERNATIONAL SEARCH REPORT

Intern al Application No
PCT/JP 01/00962

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D307/93 C07D493/08 A61K31/343 A61P33/02
//(C07D493/08,307:00,307:00)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, CHEM ABS Data, PAJ, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ORTEGA, A. ET AL: "Sphaerocephalin, a germacranolide isolated from Viguiera sphaerocephala" PHYTOCHEMISTRY (1980), 19(7), 1545-6 , XP001002830 page 1545, column 1 page 1546; example 2 ---	1,2,7
X	BOHLMANN, FERDINAND ET AL: "Naturally occurring terpene derivatives. Part 300. Eudesmanolides and diterpenes from Wedelia trilobata and an ent-kaurenic acid derivative from Aspilia parvifolia" PHYTOCHEMISTRY (1981), 20(4), 751-6 , XP001002823 page 754 --- -/--	1,2,7

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 June 2001	10/07/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Paisdor, B

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INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/JP 01/00962

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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X	SEAMAN, FRED C. ET AL: "Montanoa-terpenes. Part 5. Sesquiterpene lactones of Montanoa guatemalensis and Montanoa tomentosa subsp. xanthiifolia" PHYTOCHEMISTRY (1985), 24(9), 2003-5 , XP001002834 page 2003, column 2 ---	1,2,7
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X	WO 98 51302 A (UNIVERSITY OF WASHINGTON, USA) 19 November 1998 (1998-11-19) abstract; claims page 9, line 31 ---	1,2,7
X	BOHLMANN, FERDINAND ET AL: "Naturally-occurring terpene derivatives. Part 101. New germacrolides from Calea zacatechichi" PHYTOCHEMISTRY (1977), 16(7), 1065-8 , XP001002832 page 1067 ---	1,2,7

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MARTINEZ-VAZQUEZ, MARIANO ET AL: "The revised structure of the cytotoxic heliangolide euparhombin" J. NAT. PROD. (1991), 54(6), 1642-4 , XP001002855 page 1642 ---	1,2,7
X	KUPCHAN, S. MORRIS ET AL: "Structure-activity relationships among in vivo active germacranolides" J. PHARM. SCI. (1978), 67(6), 865-7 , XP002169692 abstract page 865, column 2 ---	1,2,7
X	GONZALEZ, ANTONIO G. ET AL: "Constituents of the Compositae. Part 36. Two sesquiterpene lactones from Centaurea canariensis" PHYTOCHEMISTRY (1978), 17(5), 955-6 , XP001002831 page 956 ---	1,2,7
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X	CHEMICAL ABSTRACTS, vol. 119, no. 9, 30 August 1993 (1993-08-30) Columbus, Ohio, US; abstract no. 91234, GONZALEZ, ANTONIO G. ET AL: "Distribution of sesquiterpene lactones in Cheirolophus from the Canary Islands" XP002169693 abstract; figures 106,107 & BIOCHEM. SYST. ECOL. (1993), 21(2), 267-70 , ---	1,2
X	CHEMICAL ABSTRACTS, vol. 98, no. 17, 25 April 1983 (1983-04-25) Columbus, Ohio, US; abstract no. 137307, ARRICK, BRADLEY A. ET AL: "Inhibition of glutathione synthesis augments lysis of murine tumor cells by sulfhydryl-reactive antineoplastics" XP002169694 abstract; figures 126,127 & J. CLIN. INVEST. (1983), 71(2), 258-67 , --- -/--	1,2,7

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Interna al Application No
PCT/JP 01/00962

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 112, no. 13, 26 March 1990 (1990-03-26) Columbus, Ohio, US; abstract no. 111628, CEA, G. ET AL: "Genotoxic effects of erioflorin acetate and erioflorin methacrylate: sesquiterpene lactones isolated from Podanthus ovatifolius Lag. (Compositae)" XP002169695 abstract; figure 120 & BULL. ENVIRON. CONTAM. TOXICOL. (1990), 44(1), 19-28 ,	1,2,7
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X	CHEMICAL ABSTRACTS, vol. 88, no. 3, 16 January 1978 (1978-01-16) Columbus, Ohio, US; abstract no. 23172, GUERRERO, C. ET AL: "Determination of the structure of euparhombin and its cytotoxic activity in two cellular lines" XP002169697 abstract; figures 124,125 & REV. LATINOAM. QUIM. (1977), 8(3), 123-7 ,	1
A	WO 93 08195 A (UNIV SYDNEY) 29 April 1993 (1993-04-29) abstract; claim 1	1-8

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nation on patent family members

Intern: l Application No
PCT/JP 01/00962

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		EP 1019044 A	19-07-2000
WO 9308195 A	29-04-1993	AU 659505 B	18-05-1995
		JP 7500325 T	12-01-1995